PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

	Thomas Dag Horn and Sandra Marchese Johnson	Examiner: Gary B. Nickol
Serial No.:	10/081,185	Group Art Unit: 1642
Filed:	February 25, 2002	Docket: 110.004US2
For:	IMMUNOTHERAPY OF EPITHELIAL TUMORS USING INTRALESIONAL INJECTION OF ANTIGENS THAT INDUCE A DELAYED TYPE HYPERSENSITIVITY REACTION	

APPELLANTS' AMENDED BRIEF ON APPEAL

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Amended Brief is presented in support of the Appeal filed August 24, 2005, from the final rejection of claims 1, 4-7, 15, 33, 36, 37, and 48-51 of the above-identified patent application, as set forth in the Final Office Action mailed May 31, 2005.

This Amended Brief is submitted in reply to the Notification of Non-Compliant Appeal Brief mailed November 14, 2005. An original Brief was filed on August 24, 2005, accompanied by a Notice of Appeal and a check in the amount of $500 to cover the small entity fees for Notice of Appeal and filing a brief in support of an appeal under 37 C.F.R. § 41.20(b)(1) and (b)(2). Appellants reserve the right to later file a Request for Oral Hearing.

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TRANSMITTAL LETTER FOR

APPELLANTS' AMENDED BRIEF ON APPEAL

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

In response to the Notification of Non-Compliant Appeal Brief mailed November 14, 2005, I am transmitting the following with this transmittal letter.

Appellants' Amended Brief on Appeal.

Return postcard.

In addition, I am enclosing copies of Case Law Cited and Relevant Art of Record and Evidence, including a Declaration under 37 C.F.R. 1.132 by Thomas Dag Horn executed 8/18/2005. All of these were enclosed with the original Appeal Brief and Notice of Appeal filed August 24, 2005. Also filed at that time was a check for $500 to cover the small entity fees for a Notice of Appeal and a Brief in Support of a Notice of Appeal under 37 C.F.R. §§ 41.20(b)(1) and 41.20(b)(2).

By: Hugh McTavish — Date: Dec. 2 2005

Hugh McTavish
Reg. No. 48,341
Phone 651-207-8270
McTavish Patent Firm
429 Birchwood Courts
Birchwood, MN 55110

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United State Postal Service with sufficient postage as first class mail, in an envelope addressed to: Mail Stop Appeal Brief - Patents, Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 2nd day of December 2005.

Hugh McTavish

APPELLANTS' BRIEF ON APPEAL

TABLE CONTENTS

1. REAL PARTY IN INTEREST

The real party in interest of the above-identified patent application is the assignee, the Board of Trustees of the University of Arkansas, Little Rock, AR.

2. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences known to Appellants' representative that will directly affect, be directly affected by, or have a bearing on the Board's decision in the pending appeal.

3. STATUS OF CLAIMS

Claims 1, 4-7, 9-12, 15-17, 33, 36-37, 40-41, and 46-51 (Appendix I) are pending in this application. Claims 9-12, 16-17, 40-41 and 46-47 stand withdrawn from consideration as drawn to a non-elected invention. Claims 1, 4-7, 15, 33, 36-37, and 48-51 stand rejected and are the subject of this appeal.

The claims withdrawn from consideration are incorrectly not listed as pending in the Examiner's Final Office Action. These claims were never cancelled and so are still pending. It is the appellants' position that claim 1 is a linking claim with respect to the withdrawn claims, and thus if claim 1 is found allowable, the withdrawn claims, which include all of the limitations of the linking claim, must be rejoined.

4. STATUS OF AMENDMENTS

The claims have not been amended since the final rejection.

5. SUMMARY OF CLAIMED SUBJECT MATTER

The invention involves intralesionally injecting antigens that cause a cutaneous delayed type hypersensitivity reaction into epithelial tumors such as warts, thereby causing a cutaneous delayed type hypersensitivity immune response in the area of the wart. The invention is based on the discovery that even when the antigens are not related to the virus that causes warts, the antigen injection leads to the production of immune cells that recognize the virus causing the wart, and the immune cells then reduce the severity of warts, including warts distant from the site

where the antigen was administered, or cure the patient of warts. (Specification, page 5, lines 5-31; page 16, Table B.)

The claims recite a pharmaceutical composition comprising at least two antigens and a pharmaceutically acceptable carrier, wherein (1) each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in a mammalian subject; (2) the composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject; and (3) one of the two antigens is a bacterial antigen and the other is a candida antigen.

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

a. Whether claims 1, 4-7, 33, 36, and 48-51 are anticipated by Bostwick, E. (U.S. Published Patent Application 2002/0009429 A1) under 35 U.S.C. § 102(e) in spite of a declaration that, as the Examiner concedes, establishes possession of the invention of administering antigens causing a delayed type hypersensitivity response to epithelial tumors to treat the tumors, including antigens unrelated to the causative agent of the epithelial tumor, before the effective date of Bostwick.

b. Whether claims 1, 4-7, 33, 36-37, and 48-51 are anticipated under 35 U.S.C. 102(e) by Clements (U.S. Patent No. 6,033,673) under a theory of inherency.

c. Whether claims 1, 4-7, 15, 33, 36-37, and 48-51 are obvious under 35 U.S.C. § 103(a) over Clements or Bostwick in further view of the Candin package insert.

For each ground of rejection which appellant contests herein that applies to more than one claim, such additional claims, to the extent separately identified and argued below, do not stand or fall together.

7. ARGUMENT

Issue (a) – Whether claims 1, 4-7, 33, 36, and 48-51 are anticipated by Bostwick, E. (U.S. Published Patent Application 2002/0009429 A1) under 35 U.S.C. § 102(e) in view of a declaration that, as the Examiner concedes, establishes possession of the invention of administering antigens causing a delayed type hypersensitivity response to epithelial tumors to treat the tumors, including antigens unrelated to the causative agent of the epithelial tumor, before the effective date of Bostwick.

The Examiner rejected claims 1, 4-7, 33, 36, and 48-51 under 35 U.S.C. § 102(e) as anticipated by Bostwick (U.S. Published Patent Application 2002/0009429 A1, filed January 29, 1999). This rejection is respectfully traversed.

In a Declaration under 37 C.F.R. § 1.131 dated 11/8/04 and filed with the response on 11/19/04 the inventor Dr. Horn states that he conceived the claimed invention of the present application before the filing date of Bostwick and diligently pursued development from conception until the filing of the patent application. The declaration of prior conception is evidenced by an attached approval letter dated before January 29, 1999, "to proceed with use of mumps and candida intradermal skin test antigens to treat human patients afflicted with Verruca vulgaris (common warts)." Dr. Horn goes on to note:

> The letter refers to the use of mumps and candida antigens. At the time of the letter, I also believed that any antigen that induced a cutaneous delayed-type hypersensitivity response, including bacterial antigens, would successfully treat warts and other benign epithelial tumors. At the time of this letter from Dr. Faas and at the time of submitting the protocol that the letter refers to, I planned to combine two or more antigens in a single composition to be administered for treatment of warts and other epithelial tumors. The compositions with two or more antigens that I had conceived and planned to use included compositions containing mumps and candida antigens, as well as compositions containing candida and bacterial antigens.

The Examiner asserts that this only "establishes conception of a composition comprising mumps and candida antigens to treat . . . common warts," (Office Action mailed Feb. 2, 2005) but does not establish conception of a composition comprising candida and bacterial antigens, each of which induces or is capable of inducing a cutaneous delayed type hypersensitivity reaction.

Appellants disagree. The declaration establishes conception of a composition containing any antigen or antigens that induce or are capable of inducing a cutaneous delayed type hypersensitivity reaction to treat common warts. Common warts are caused by human papilloma virus (HPV) (specification, page 1, lines 19-20), not by either candida or mumps. This was obviously understood by Dr. Horn when he planned to use candida and mumps antigens to treat warts. Candida is a fungus and mumps is a virus, so the two antigens have little in common other than both having a high prevalence of reactivity in humans that results in the elicitation of a DTH response (specification, page 5, lines 5-7). It strains credulity to argue that when Dr. Horn planned to use candida and mumps antigens, which he knew were unrelated to each other and

unrelated to the virus that causes warts, to treat warts, he did not at that time believe that other antigens unrelated to HPV, such as bacterial antigens, would also be effective to treat warts. And Dr. Horn has sworn in his declaration that he did believe at that time that any antigen, including bacterial antigens, that induced a cutaneous delayed type hypersensitivity reaction could be used to treat warts. Thus, the declaration establishes that Dr. Horn and his co-inventor, had conceived the use of any antigen or antigens that induce or are capable of inducing a cutaneous delayed type hypersensitivity reaction to treat common warts, including as is recited in the present claims, a bacterial antigen and a candida antigen.

The Examiner states that the "declaration must establish possession of either the whole invention claimed or something falling within the claim (such as a species of a claimed genus), in the sense that the claim as a whole reads on it," citing *In re Tanczyn*, 347 F.2d 830, 146 U.S.P.Q. 298 (C.C.P.A. 1965), and that Dr. Horn's declaration does neither (Office Action mailed Feb. 2, 2005). First, as argued above, Appellants contend that Dr. Horn's declaration does establish possession before the filing date of Bostwick of use of a bacterial antigen and a candida antigen to treat warts, where both antigens induce a DTH response, as well as any other antigen inducing a DTH response.

Furthermore, "Rule 131 requires applicant to make oath to facts showing completion 'of the invention.' That requirement does not mean affiant must show a reduction to practice of every embodiment of the invention." *In re Hostettler*, 356 F.2d 562 (C.C.P.A. 1966). The invention here is the use of an antigen to treat an epithelial tumor, such as warts, by injection into the epithelial tumor, where the antigen induces or is capable of inducing a cutaneous delayed type hypersensitivity (DTH) response. Dr. Horn's Declaration shows possession of that invention before the filing date of Bostwick.

The facts here show some parallels to those of *In re Stryker*, 435 F.2d 1340 (C.C.P.A. 1971). In *Stryker*, the applicant claimed a "process for removing polypropylene diluent from a suspension consisting essentially of from about 50%-60% by weight polypropylene . . ." *Id.* at 1340. The applicant attempted to remove a cited reference with a Rule 131 affidavit. The Board of Patent Appeals and Interferences held that the affidavit was deficient because it did not demonstrate possession of the particular weight percentages recited in the claim. *Id.* at 1341. The C.C.P.A. overturned the rejection. The C.C.P.A. first distinguished *Tanczyn*, the case also cited by the Examiner here, as concerning a situation where "the subject matter shown in [both]

the reference and the affidavit was so different from the claimed invention that the claims were unobvious and patentable over the reference." *Id.* at 1341. The Court in *Stryker* then held:

> "To hold that Harban is not removed by the showing here presented would lead to an anomalous result, i.e., if appellant broadened his claims by deleting the weight limitations so as to read literally on Harban, Harban would not be available as a reference against such broadened claims because appellant's antedating affidavit would be satisfactory in every respect. It cannot be the law that the same affidavit is insufficient to remove the same reference applied against the slightly narrower claims presented here." *Id.* at 1341-1342.

The facts here are even stronger. The Examiner concedes that Dr. Horn's Declaration establishes possession of a pharmaceutical composition comprising a mumps antigen and a candida antigen. The originally filed claim 1, recited "A pharmaceutical composition for treating an epithelial tumor in a subject comprising at least two antigens and a pharmaceutically acceptable carrier, wherein each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in the subject." That originally filed claim 1 reads on the embodiment of the invention that the Examiner concedes Appellants possessed before the Bostwick filing date. But the Examiner required that Appellants narrow claim 1 in a restriction requirement to make searching easier. Now the Examiner rejects use of the Declaration to remove Bostwick on the basis that the narrowed claim 1 no longer reads on the embodiment shown by the Declaration. The broader claim that would read on the embodiment shown by Dr. Horn's Declaration is, in fact, not just a hypothetical claim as was the case in *Stryker* but the originally filed claim 1 reciting a pharmaceutical composition comprising at least two antigens. This claim was narrowed to recite a pharmaceutical composition comprising a bacterial antigen and a candida antigen, solely in response to the Examiner's restriction requirement. To paraphrase the court in *Stryker*, it cannot be the law that the Examiner can demand that the claims be narrowed to make searching easier, and then assert that the Appellants' showing of possession with a Rule 131 Declaration, while it would have been sufficient to remove the reference with the original claims, is not sufficient with the narrower claims that the Examiner required in a restriction requirement.

Alternatively, Appellants' showing of a reduction to practice of a pharmaceutical composition comprising a mumps antigen and/or a candida antigen for treating warts establishes possession of a pharmaceutical composition comprising a bacterial antigen and a candida antigen for treating warts, wherein each of said antigens induces or is capable of inducing a cutaneous

delayed type hypersensitivity response in a mammalian subject, because the latter composition is obvious in view of the former. This rule of obviousness in considering Rule 131 Declarations was elucidated in *In re Spiller* (500 F.2d 1170 (C.C.P.A. 1974)). *In re Spiller* is another case of an appeal from a rejection by the Board of an attempt with a Rule 131 Declaration to swear behind a cited reference. As in *Stryker*, the Board rejection was on the basis that the Declaration did not establish possession of all the limitations of the claims, and as in *Stryker* the C.C.P.A. overturned the Board decision. The C.C.P.A. held in *In re Spiller* that "for the purpose of antedating [a reference] under Rule 131, it is sufficient that appellant has shown a reduction to practice of his basic invention, which showing will also suffice as to claims differing therefrom only in details which are obvious to one of ordinary skill in the art." *In re Spiller*, at 1178.

Mumps antigen and candida antigen are both unrelated to the papilloma virus that causes common warts. They have little in common with each other besides the fact that both induce a delayed type hypersensitivity response in many human subjects because a large portion of the population has sensitivity to each. One antigen is viral and the other fungal. From those facts, which were known at the time of the Appellants' invention, it would have been obvious to one of ordinary skill in the art, in view of Appellants' invention of using mumps antigen and candida antigen to treat warts, that any antigen which induces or is capable of inducing a delayed type hypersensitivity response could be used to treat warts.

Appellants' showing of a reduction to practice of a pharmaceutical composition comprising a mumps antigen and/or a candida antigen for treating warts renders obvious and thus establishes possession also of the presently claimed pharmaceutical composition comprising a bacterial antigen and a candida antigen for treating a benign epithelial tumor caused by a papilloma virus (e.g., warts), wherein each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in a mammalian subject.

Thus, the Rule 131 Declaration of Dr. Horn establishes possession of the invention before the filing date of Bostwick. First, Dr. Horn states in his Rule 131 Declaration that he conceived the use of any antigen, including a bacterial and candida antigen, that causes a cutaneous DTH response to treat warts before the filing date of Bostwick, and was diligent in developing the invention until the patent application was filed. This is supported by a letter from Dr. Fred Faas approving his use of candida and mumps antigens to treat warts in human patients dated before

the filing date of Bostwick. This establishes possession of the presently claimed invention before the filing date of Bostwick.

If Dr. Horn's statement in his Rule 131 Declaration that he had conceived of use of a composition containing bacterial and candida antigens before the filing date of Bostwick is disbelieved or somehow discounted, it is still admitted by the Examiner that his Rule 131 Declaration establishes possession of a pharmaceutical composition comprising mumps and candida antigens to treat common warts. This falls within the scope of the originally filed claim 1 of the present application reciting "A pharmaceutical composition for treating an epithelial tumor in a subject comprising at least two antigens and a pharmaceutically acceptable carrier, wherein each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in the subject." It thus would suffice to establish possession of that claim and swear behind Bostwick if that claim had not been amended under the rule of *In re Tanczyn* (347 F.2d 830, 146 U.S.P.Q. 298 (C.C.P.A. 1965)) that the declaration overcomes the reference if it establishes possession of either the whole invention claimed or something falling within the claim (such as a species of a genus) in the sense that the claim as a whole reads on it (M.P.E.P. 715.02). But claim 1 was amended to recite a pharmaceutical composition comprising a bacterial antigen and a candida antigen solely in response to the Examiner's restriction requirement to make the search easier. Under the rule of *In re Stryker*, the Examiner cannot demand that the claims be narrowed to make searching easier, and then assert that Appellants' showing of possession with a Rule 131 Declaration, while it would have been sufficient to remove the reference with the original claims is not sufficient with the narrower claims that the Examiner required in a restriction requirement.

Finally, even if both of those theories are rejected, Appellants' showing of a reduction to practice of a pharmaceutical composition comprising a mumps antigen and/or a candida antigen for treating warts establishes possession of a pharmaceutical composition comprising a bacterial antigen and a candida antigen for treating warts, wherein each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in a mammalian subject, because the latter composition is obvious in view of the former.

In all three of these ways, the Rule 131 Declaration of Dr. Horn establishes possession of the presently claimed invention before the filing date of Bostwick and removes Bostwick as a

reference under 35 U.S.C. § 102(e). This obviates the rejection of the claims under 35 U.S.C. § 102(e) over Bostwick.

Issue b – Whether claims 1, 4-7, 33, 36-37, and 48-51 are anticipated under 35 U.S.C. 102(e) by Clements (U.S. Patent No. 6,033,673) under a theory of inherency.

Claims 1, 4-7, 33, 36-37, and 48-51 were rejected under 35 U.S.C. § 102(e) as anticipated by Clements (U.S. Patent No. 6,033,673, filed March 18, 1998). This rejection is respectfully traversed.

Inherency theory of rejection and burden of proof.

The rejection over Clements is based on a theory of inherency.

The pending claims recite "A pharmaceutical composition comprising at least two antigens and a pharmaceutically acceptable carrier, wherein (1) each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in a mammalian subject; (2) the composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject; and (3) one of the two antigens is a bacterial antigen and the other is a candida antigen." The Examiner has noted, correctly, that the claims require that each antigen be capable of inducing a cutaneous DTH response in the pharmaceutical composition, not on its own, free of the pharmaceutical composition (page 5 of Office Action mailed Feb. 2, 2005).

Clements discloses a novel mutant of *E. coli* heat labile enterotoxin modified by two amino acid substitutions and designated LT(R192G/L211A) (abstract). It discloses that the mutant enterotoxin can be administered in conjunction with any biologically relevant antigen or vaccine, such that an increased immune response to the antigen or vaccine is achieved (col. 9, lines 36-41). It discloses that the mutant enterotoxin and antigen can be administered simultaneously in a pharmaceutical composition (col. 9, lines 43-45). It discloses that many antigens may be used in the invention, including antigens from pathogenic fungi, and specifically including *Candida albicans* (col. 10, lines 27-29). It discloses that the mutant enterotoxin promotes the production of serum and/or mucosal antibodies as well as cell-mediated immune responses against antigens that are simultaneously administered with the mutant enterotoxin (col. 9, lines 6-10). It refers to the mutant enterotoxin as an adjuvant (abstract).

The Examiner asserts that Clements's disclosure of use of its specific *E. coli* mutant enterotoxin as an adjuvant in a pharmaceutical composition with specific antigens including *Candida albicans* constitutes disclosure of a pharmaceutical composition comprising a bacterial antigen and a candida antigen, wherein each antigen induces or is capable of inducing a cutaneous DTH response and the composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject. But Clements says nothing about its composition treating a benign epithelial tumor, and it does not disclose that its *E. coli* mutant enterotoxin is an antigen at all in the pharmaceutical composition, or that it more specifically induces or is capable of inducing a cutaneous DTH response in the pharmaceutical composition disclosed. Thus, this reference can only anticipate the present claims if these traits are inherent traits of the composition disclosed in Clements. The Examiner apparently agrees, since he concedes that "the issues of inherency apply" (page 7 of the Office Action mailed May 31, 2005).

To rely on a theory of inherency to support a 35 U.S.C. § 102 rejection, the M.P.E.P. states that "the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." M.P.E.P. § 2112, citing *Ex parte Levy*, 17 U.S.P.Q.2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in the original). "[T]hat a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic." M.P.E.P. 2112, citing *In re Rijckaert*, 9 F3d 1531, 1534 (Fed. Cir. 1993) (emphasis in the original).

In contradiction to this case law and statements in the M.P.E.P. on the burden of proof in making out a rejection on the basis that a characteristic is inherent in the prior art, the Examiner attempts to shift the burden to the Appellants to prove a negative. In the Office Action mailed February 2, 2005, the Examiner states without evidence or reasoning, "each of the antigens disclosed by Clements inherently induces or is capable of inducing a cutaneous delayed type hypersensitivity response. . . . In the absence of evidence the contrary, the burden is on the applicant to prove that the claimed product is different from those taught by the prior art and to establish patentable differences." (Office Action mailed February 2, 2005, pages 4-5.) First, the Examiner simply states without evidence or reasoning that inducing a DTH response is an inherent property of the mutant enterotoxin of Clements in the compositions of Clements, and

then states that the burden is on the Appellants to prove otherwise. To support this burden shifting, he cites *In re Best,* 562 F.2d 1252 (C.C.P.A. 1977) and *Ex parte Gray,* 10 U.S.P.Q.2d 1922 (Bd. Pat. App. Int. 1989).

Both *In re Best* and *Ex parte Gray* concern claims that were functionally product-by-process claims and were evaluated under a product-by-process standard. Claim 1 in Best recited a crystalline zeolitic aluminosilicate having certain properties. *In re Best,* 562 F.2d at 1252. Claim 3 recited a process for preparing the product recited in claim 1. *In re Best,* 562 F.2d at 1253. The court cited a reference wherein

> [a]ll the positive process limitations are expressly disclosed except for the functionally expressed rate of cooling. However, there is nothing to indicate that this rate of cooling in any way differs from the normal rate resulting from removal of the heat source. Thus, the examiner's conclusion that those parameters of the resultant product which are recited in the appealed claims but are not expressly disclosed in the reference would be inherent is a reasonable one, absent convincing evidence to the contrary. *In re Best*, at 1254.

Ex parte Gray also involved claims evaluated as product-by-process claims. "While the present claims are drafted in the form of a compound or a composition, the rationale underlying appellants' arguments is founded on the proposition that the claims are directed to a product-by-process. In any event, we are convinced that the legal philosophy employed in rejections involving products-by-process should be employed with respect to the claims before us." *Ex parte Gray*, 10 U.S.P.Q.2d at 1924.

The burden of proof for the Patent Office in making out a rejection for anticipation or obviousness of product-by-process claims is lower, according to the M.P.E.P. "The Patent Office bears a lesser burden of proof in making out a case of *prima facie* obviousness for product-by-process claims because of their peculiar nature." M.P.E.P. § 2113, quoting *In re Fessmann*, 489 F.2d 742, 744, 180 U.S.P.Q. 324, 326 (C.C.P.A. 1974).

Product-by-process claims are directed, typically, to a product that is nearly identical to a prior art product known to the inventors, differing only in the manner the product is produced. The differing method of production is alleged to create different properties in the product. Both *In re Best* and *Ex parte Gray* concerned products that were nearly identical to prior art products known to the inventors, but were prepared by a new process. *Ex parte Gray* concerned claims directed to human β nerve growth factor produced by recombinant means. *Ex parte Gray*, 10 U.S.P.Q.2d at 1923. The protein was already known, and the issue was whether producing the

protein by recombinant means created a patentably distinct product. *In re Best* concerned a zeolitic aluminosilicate useful as a catalyst. *In re Best*, 562 F.2d at 1252. Similar zeolitic aluminosilicates useful as catalysts were already known. *Id.* at 1253. The claimed catalyst differed from the prior art catalysts only in the rate of cooling used to produce it. *Id.* at 1254. Where the only difference between a claimed product and a prior art product is the way it is produced, the board in *Ex parte Gray* and the court in *In re Best* have held that it is appropriate to shift the burden to the applicant to demonstrate that the different method of production creates a patentable difference in the product.

But the present claims are not product-by-process claims. So the lesser product-by-process standard is not appropriate. The appropriate standard is the standard for inherency rejections, as described above.

In the last Office Action, the Examiner quotes *In Re Best*, "the claiming of a new use, new function or unknown property which is inherently present in the prior art does not necessarily make the claim patentable." (Page 6 of the final Office Action mailed May 31, 2005, emphasis added, quoting *In re Best* at 1252.) The Examiner is making Appellants' case: the question for the Board is whether the properties recited in the present claims are inherently present in the prior art. But again, to establish that the recited properties are inherently present in the prior art and to substantiate a rejection on the basis of inherency, "the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teaching of the applied art." M.P.E.P. § 2112, citing *Ex parte Levy*, 17 U.S.P.Q.2d 1461, 1464 (Bd. pat App. & Inter. 1990) (emphasis in the original).

Applying the standard for an inherency rejection to Clements and the present claims.

The question for the board is whether the composition disclosed in Clements containing the *E. coli* heat labile LT(R192G/L211A) mutant enterotoxin and an antigen, which may be a *Candida albicans* antigen, is necessarily a composition in which (1) the *E. coli* heat labile LT(R192G/L211A) is an antigen, (2) both the mutant enterotoxin and the *Candida albicans* antigen are antigens that induce or are capable of inducing a cutaneous DTH response in a mammalian subject in the composition disclosed, and (3) the composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject. If the *E. coli*

mutant enterotoxin when it is in the composition disclosed in Clements (1) is not necessarily an antigen, or (2) does not necessarily induce, or is not necessarily capable of inducing, a cutaneous DTH response, then Clements does not anticipate the present claims.

The *E. coli* mutant enterotoxin of Clements is not necessarily antigenic in the composition disclosed therein and is not necessarily capable of inducing a cutaneous DTH response in the composition disclosed therein because (1) not all proteins are antigenic in all compositions, (2) not all antigens are capable of inducing a DTH response, and (3) even those antigens that are capable of inducing a DTH response in some compositions may not induce the response in other compositions.

Clements describes its mutant *E. coli* enterotoxin as an adjuvant (abstract), not as an antigen. An adjuvant is "a vehicle used to enhance antigenicity, e.g., a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is absorbed . . ." (Stedman's Medical Dictionary, 27th edition.) In contrast, an antigen is "[a]ny substance that, as a result of coming in contact with appropriate cells, induces a state of sensitivity and/or immune responsiveness after a latent period (days to weeks) and that reacts in a demonstrable way with antibodies and/or immune cells of the sensitized subject in vivo or in vitro." (Stedman's Medical Dictionary, 27th ed.) Thus, to be an antigen, a substance must induce an immune response against itself. If it promotes an immune responses against another substance in the composition, it would be an adjuvant, which is how Clements describes the *E. coli* mutant enterotoxin, and not necessarily an antigen.

Not all proteins in all compositions are antigenic. Dr. Horn declares in the enclosed Declaration[1] under 37 C.F.R. § 1.132 executed 8/18/05, "[M]any foreign substances presented in certain compositions produce no immune response. The purpose of adjuvants such as Freund's complete adjuvant, is to enhance immune response to other potentially antigenic substances in the composition. Often an antigen can elicit a large immune response when presented with an adjuvant, but no detectable immune response when presented without an adjuvant. Thus, foreign substances that are potentially antigenic do not behave as antigens – that is, they do not induce an immune response – in certain formulations and with certain modes of presentation."

[1] The Rule 132 Declaration of Dr. Horn was not presented earlier because literature citations were relied upon to show that not all antigens induce or are capable of inducing a cutaneous DTH response in the Amendment filed March 5, 2005. The Examiner made the next Office Action final and indicated that Applicants' arguments based on

Even if a substance is antigenic in a particular composition, not all substances that are antigenic induce or are capable of inducing a cutaneous DTH response. Dr. Horn states in his Rule 132 Declaration: "[W]hen antigens do induce an immune response, the immune response can be in many forms, most of which are not a delayed type hypersensitivity response." This is evidenced not only by Dr. Horn's Rule 132 Declaration, but also by numerous statements in textbooks of immunology and in the published scientific literature. For instance, one textbook states: "A major question still remains as to the factors involved in determining whether cellular [e.g., a DTH response] or humoral immunity will develop in response to a certain antigen." (Nieuwenhuis, P., pp. 3-32, at page 27, in Marsh, J.A. et al. eds., *The Physiology of Immunity*, CRC Press, Boca Raton, FL, 1996.)

Dr. Horn cites two other published papers that show that not all antigens induce or are capable of inducing a DTH response in all compositions. Lichtenwalner et al., 2004, *Infection and Immunity*, 72:1159-1161, discloses testing several antigens from Chlamydia for induction of a delayed type hypersensitivity (DTH) response. Of the antigens, tested, only heat shock protein 60 induced a DTH response, while killed whole organisms, outer membrane protein, and heat shock protein 10 did not. To take another example, Shibata et al., 2001, *Infection and Immunity* 69:6123-6130, reports that immunization of mice with MPD-59 mycobacterial protein without chitin induces certain immune response including IgE production and Th2 cells producing IL-4, IL-5, and IL-10, but does not induce Th1 cells or a delayed type hypersensitivity response (abstract). (Paragraph 7, Dr. Horn's Rule 132 Declaration.)

Thus, it is very clear that not all antigens induce or are capable of inducing a DTH response, especially not in all compositions. Furthermore, which antigens induce a DTH response cannot be predicted in advance, or the authors of the Lichtenwalner et al. and Shibita et al. papers would not have conducted their studies.

Clements discloses compositions containing a mutant enterotoxin and an antigen, and that administration of these compositions induces an immune response to the antigen (col. 9, lines 36-41). Clements does not disclose that any immune response directed to the mutant enterotoxin is generated. Much less does it disclose that specifically a cutaneous DTH response to the mutant enterotoxin is generated. Since not all antigens in all compositions induce a DTH response, and

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published literature were attorney statements that are not evidence and must be supported by an appropriate affidavit or declaration (page 8 of the Office Action mailed May 31, 2005).

Clements does not disclose that any DTH response to the mutant enterotoxin of its compositions is generated, it is therefore not a necessarily inherent characteristic of the compositions disclosed in Clements that the mutant enterotoxin administered in those compositions induces or is capable of inducing a cutaneous DTH response against itself. Thus, Clements does not disclose a composition containing a candida antigen and a bacterial antigen, each of which induces or is capable of inducing a cutaneous delayed type hypersensitivity response in a mammalian subject.

Dependent claims

Even if the mutant enterotoxin of Clements were inherently an antigen that is capable of inducing a cutaneous DTH response in the compositions disclosed therein, claims 48-51 are still novel over Clements.

Clements discloses a new mutant *E. coli* enterotoxin molecule (column 6, lines 21-28). Since it is a new engineered molecule generated by site-directed mutagenesis (column 12, lines 59-61), no human or other mammal has been exposed to it and no human or other mammal would be expected to have a preexisting sensitivity to it. Thus, claims 48-49, reciting that humans have a preexisting sensitivity to each of the antigens such that each of the antigens, when injected intradermally into a human subject, induces a cutaneous delayed type hypersensitivity response in at least some human subjects (claim 48) or in most healthy human subjects (claim 49) are novel over Clements.

The Examiner has replied to this argument by stating that Clements discloses administration as boosters wherein the initial administration of the toxin and antigen is followed by a boost which may comprise the antigen alone or in combination with enterotoxin. (Final Office Action page 9, citing Clements column 9, lines 50-65.) This interpretation renders the limitation of preexisting sensitivity in claims 48 and 49 meaningless. If preexisting sensitivity to the presently claimed compositions exists where the compositions are first given to a subject to create preexisting sensitivity and then given as a booster, then the limitation is meaningless. Any antigenic composition can be given to a naive subject, never previously exposed to the antigen, to create sensitivity. But if the suggestion of administering an antigenic composition in a series of two or more injections constitutes preexisting sensitivity to the antigens contained in the composition, than the limitation is meaningless. All compositions containing antigens then are compositions containing antigens wherein humans have a preexisting sensitivity to each of said

antigens. In order for the claim limitation to be a limitation at all, it must mean that humans have a preexisting sensitivity to the antigens before being exposed to the composition. Thus, this disclosure of Clements does not establish that the mutant enterotoxin of Clements is an antigen humans have a preexisting sensitivity to.

Claim 49 recites the pharmaceutical composition of claim 48 wherein each of said antigens induces a cutaneous delayed type hypersensitivity response in most healthy human subjects. Thus, this claim, depending on claim 48, includes the limitation of humans having a preexisting sensitivity to each of said antigens and the limitation that each of said antigens induces a cutaneous delayed type hypersensitivity response. Despite rejecting it, the Examiner did not comment on claim 49, but even if Clements' disclosure of administering its compositions as a boost constitutes "preexisting sensitivity" to the antigens, Clements does not suggest sensitizing most humans to its mutant enterotoxin, and so it would not be the case that the mutant enterotoxin induces a delayed type hypersensitivity response in most healthy human subjects.

Claim 50 recites the pharmaceutical composition of claim 1 wherein each of said antigens has a high prevalence of reactivity in humans or another mammal to induce a cutaneous delayed type hypersensitivity response. Again, since the enterotoxin adjuvant of Clements is a novel engineered molecule, it is not likely that humans or other mammals have a high prevalence of reactivity to it. Furthermore, no evidence has been introduced that even wild type *E. coli* enterotoxin has a high prevalence of reactivity in humans or another mammal to induce a cutaneous delayed type hypersensitivity response. Thus, claim 50 also is novel over Clements. The Examiner offered no evidence or reasoning to rebut this argument for the patentability of claim 50 in the Final Office Action.

Claim 51 recites the pharmaceutical composition of claim 1 wherein each of said antigens is an antigen from a naturally occurring infectious agent. The enterotoxin of Clements is a new engineered molecule generated by site-directed mutagenesis (column 12, lines 59-61). Thus, it is not an antigen from a naturally occurring infectious agent. Again, this argument was raised previously, and the Examiner rejected this claim in the Final Office Action, but he offered no rationale for the rejection and no response to the argument.

Issue c – Whether claims 1, 4-7, 15, 33, 36-37, and 48-51 are obvious under 35 U.S.C. § 103(a) over Clements or Bostwick in further view of the Candin package insert.

Claims 1, 4-7, 33, 36-37, and 48-51 were rejected as obvious under 35 U.S.C. § 103(a) over Clements (U.S. Patent No. 6,033,673) or Bostwick (U.S. Published Patent Application 2002/0009429) in further view of the CANDIN® package insert. This rejection is respectfully traversed.

Bostwick is removed as prior art for the reasons argued under **Issue a** in this brief.

Three criteria must be met in order to establish a *prima facie* case of obviousness. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. M.P.E.P. § 2142, citing *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

With the removal of Bostwick as prior art by Dr. Horn's Rule 131 Declaration, as argued under **Issue a** in this brief, the rejection becomes a rejection over Clements in further view of the CANDIN® package insert.

The teachings of Clements and its deficiencies with respect to the present claims are discussed above under **Issue b**. Specifically, Clements discloses a composition containing the engineered *E. coli* heat labile LT(R192G/L211A) mutant enterotoxin and an antigen, which may be a *Candida albicans* antigen. But the mutant enterotoxin is not disclosed to be itself antigenic in the compositions. It is also not disclosed to induce or be capable of inducing a cutaneous DTH response in a mammalian subject in the compositions disclosed. It is well known and established by Dr. Horn's Rule 132 Declaration executed August 18, 2005, and by Lichtenwalner et al., 2004, *Infection and Immunity*, 72:1159-1161 and Shibata et al., 2001, *Infection and Immunity* 69:6123-6130, both cited in Dr. Horn's Declaration and above in this brief, and by Nieuwenhuis, cited above in this brief, that potentially antigenic substances may not be antigens in a particular composition, and if antigenic may not induce a delayed type hypersensitivity response. Accordingly, Clements does not disclose a composition that contains a candida antigen and a bacterial antigen, wherein each of said antigens induces or is capable of inducing a cutaneous DTH response in a mammalian subject, and wherein the composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject.

Furthermore, Clements discloses its *E. coli* mutant enterotoxin is an adjuvant, not an antigen (abstract). Thus, it does not disclose or suggest the desirability of using the enterotoxin as an antigen – that is, of generating an immune response against the enterotoxin itself. Much less does it suggest the desirability of generating specifically a cutaneous DTH response to the enterotoxin. It also does not disclose or suggest that its compositions are capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject. Accordingly, Clements does not disclose or suggest all the elements of the presently claimed invention.

The CANDIN® package insert does nothing to remedy these deficiencies of Clements with respect to the present claims. The CANDIN® package insert describes the product as a Skin Test Antigen for Cellular Hypersensitivity made from the culture filtrate and cells of two strains of *Candida albicans*, a fungus (Description). It discloses it is indicated for use for detecting DTH by intracutaneous (intradermal) testing (Indications and Usage).

The CANDIN® package insert does not disclose or suggest combining the candida antigens with bacterial antigens that induce a cutaneous DTH response in a single pharmaceutical composition. It also does not disclose or suggest that the CANDIN® composition or any other composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject.

Thus, neither Clements or the CANDIN® package insert discloses or suggests combining a candida antigen and a bacterial antigen in a single pharmaceutical composition wherein both antigens induce or are capable of inducing a cutaneous DTH response in the compositions. Furthermore, neither discloses or suggests a composition that is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject. Thus, the references do not teach or suggest all the elements of the present claims.

The references also provide no suggestion or motivation to modify the reference teachings to arrive at the presently claimed invention. The CANDIN® package insert discloses the existence of candida antigens and that they can be used to detect a cutaneous DTH response. Clements discloses use of a particular mutant *E. coli* enterotoxin as an adjuvant to enhance immune response to particular antigens. Clements discloses candida antigen as one antigen suitable for use in its compositions. Since Clements discloses the existence of the candida antigens, the CANDIN® package insert really adds nothing to the disclosure of Clements. Thus,

the references do not provide a suggestion or motivation to modify reference teachings to arrive at the presently claimed invention.

Clements and the CANDIN® package insert also do not establish a reasonable expectation of success in creating a pharmaceutical composition comprising at least two antigens and a pharmaceutically acceptable carrier, wherein each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in a mammalian subject, the composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject, and one of the two antigens is a bacterial antigen and the other is a candida antigen, because it was unknown until Appellants' invention that a composition containing a bacterial antigen and a candida antigen, wherein each of the antigens induces or is capable of inducing a cutaneous DTH response, could treat a benign epithelial tumor caused by a papilloma virus.

Accordingly, the combination of Clements and the CANDIN® package insert does not establish even one of the three requirements for a *prima facie* case of obviousness. Furthermore, the Examiner has not pointed to the particular features of Clements and the CANDIN® package insert that are alleged to establish a *prima facie* case of obviousness. The Examiner simply makes conclusory statements such as "Applicant's claimed invention fails to patentably distinguish over the state of the art represented by the cited references taken in combination," (page 10, Final Office Action mailed May 31, 2005) without pointing specifically to where all the elements of the present claims are taught in the two references, what teaching or suggestion is provided to combine or modify reference teachings, or what in the cited references establishes a reasonable likelihood of success of creating a composition that could treat a benign epithelial tumor caused by a papilloma virus.

For the reasons advanced above, Appellants respectfully contend that each claim is patentable. Therefore, reversal of all rejections is courteously solicited.

Respectfully submitted,

THOMAS DAG HORN ET AL

By Their Representatives,

McTavish Patent Firm
429 Birchwood Courts
Birchwood, MN 55110
651-207-8270

Date Dec 2, 2005 By: Hugh McTavish

Hugh McTavish
Reg. No. 48,341

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient first class postage, in an envelope addressed to: Mail Stop Appeal Brief - Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 2 day of December, 2005.

Hugh McTavish

Hugh McTavish

APPENDIX I

The Claims on Appeal

What is claimed is:

1. (Previously presented) A pharmaceutical composition comprising at least two antigens and a pharmaceutically acceptable carrier, wherein

each of said antigens induces or is capable of inducing a cutaneous delayed type hypersensitivity response in a mammalian subject;

the composition is capable of treating a benign epithelial tumor caused by a papilloma virus in a mammalian subject; and

one of the two antigens is a bacterial antigen and the other is a candida antigen.

2-3. Canceled.

4. (Previously presented) The pharmaceutical composition of claim 1 wherein the composition is capable of treating a benign epithelial tumor caused by a human papilloma virus in a human subject.

5. (Previously presented) The pharmaceutical composition of claim 1, wherein said benign epithelial tumor is a verruca, a condyloma, bowenoid papulosis, a laryngeal papilloma, or a epidermodysplasia verruciformis.

6. (Original) The pharmaceutical composition of claim 5, wherein said verruca is verruca vulgaris, verruca plantaris, verruca palmeris or verruca plana.

7. (Original) The pharmaceutical composition of claim 1, wherein said antigens are antigenic determinants, haptens or epitopes of said antigens and are responsible for inducing said delayed type hypersensitivity response in the subject.

8. Canceled.

9. (Withdrawn) The pharmaceutical composition of claim 1 wherein the composition further comprises a trichophyton antigen, a mumps antigen, or a combination thereof.

10. (Withdrawn) The pharmaceutical composition of claim 9, wherein said antigens are a combination of candida, trichophyton and mumps.

11. (Withdrawn) The pharmaceutical composition of claim 1, further comprising at least one cytokine or colony stimulating factor into said tumor.

12. (Withdrawn) The pharmaceutical composition of claim 11, wherein said colony stimulating factor is granulocyte macrophage colony stimulating factor and said cytokine is interferon-α, interferon-β, interferon-γ, interleukin-2 or interleukin-12.

13-14. Canceled.

15. (Original) A kit comprising at least one container, a hypodermic needle or a high pressure injection device, and the pharmaceutical composition of claim 1.

16. (Withdrawn) A kit of claim 15, further comprising at least one container, a hypodermic needle or a high pressure injection device comprising at least one additional pharmaceutical composition comprising at least one cytokine or colony stimulating factor into said tumor.

17. (Withdrawn) A kit comprising at least one container, a hypodermic needle or a high pressure injection device comprising the pharmaceutical composition of claim 11.

18-32. Canceled.

33. (Previously presented) The pharmaceutical composition of claim 1, wherein said pharmaceutical composition does not contain an immunogenic additive other than said antigens.

34-35. Canceled.

36. (Previously presented) The pharmaceutical composition of claim 1, wherein one of said antigens is an allergenic *Candida albicans* extract for intradermal testing.

37. (Previously presented) The pharmaceutical composition of claim 36, wherein said allergenic *Candida albicans* extract for intradermal testing is the *Candida albicans* Skin Test Antigen.

38-39. Canceled.

40. (Withdrawn) The pharmaceutical composition of claim 10, wherein said candida antigen is an allergenic *Candida albicans* extract for intradermal testing.

41. (Withdrawn) The pharmaceutical composition of claim 40, wherein said allergenic *Candida albicans* extract for intradermal testing is the *Candida albicans* Skin Test Antigen.

42-45. Canceled.

46. (Withdrawn) The pharmaceutical composition of claim 10, wherein said candida antigen is an allergenic *Candida albicans* extract, said mumps antigen is an allergenic Mumps Skin Test Antigen and said trichophyton antigen is an allergenic trichophyton extract.

47. (Withdrawn) The pharmaceutical composition of claim 46, wherein said allergenic *Candida albicans* extract for intradermal testing is the *Candida albicans* Skin Test Antigen.

48. (Previously presented) The pharmaceutical composition of claim 1 wherein humans have a preexisting sensitivity to each of said antigens such that each of said antigens, when injected intradermally into a human subject, induces a cutaneous delayed type hypersensitivity response in at least some human subjects.

49. (Previously presented) The pharmaceutical composition of claim 48 wherein each of said antigens induces a cutaneous delayed type hypersensitivity response in most healthy human subjects.

50. (Previously presented) The pharmaceutical composition of claim 1 wherein each of said antigens has a high prevalence of reactivity in humans or another mammal to induce a cutaneous delayed type hypersensitivity response.

51. (Previously presented) The pharmaceutical composition of claim 1 wherein each of said antigens is an antigen from a naturally occurring infectious agent.

APPENDIX II

Relevant Art of Record and Evidence

Clements, U.S. Patent No. 6,033,673. Cited in the Final Office Action mailed May 31, 2005.

Bostwick, U.S. Published Patent Application No 2002/009429. Cited in the Final Office Action mailed May 31, 2005.

CANDIN® Package Insert. Cited in the Final Office Action mailed May 31, 2005.

Nieuwenhuis, P., pp. 3-32, in Marsh, J.A. et al. eds., *The Physiology of Immunity*, CRC Press, Boca Raton, FL, 1996. Entered with the Amendment and Reply filed March 5, 2005.

Lichtenwalner et al., 2004, *Infection and Immunity*, 72:1159-1161. Submitted with the Appeal Brief filed August 24, 2005.

Shibata et al., 2001, *Infection and Immunity* 69:6123-6130. Submitted with the Appeal Brief filed August 24, 2005.

Definition of "Adjuvant," Stedman's Medical Dictionary, 27th edition, 2000, Lippincott, Williams & Wilkins, Philadelphia. Submitted with the Appeal Brief filed August 24, 2005.

Definition of "Antigen," Stedman's Medical Dictionary, 27th edition, 2000, Lippincott, Williams & Wilkins, Philadelphia. Entered with the Amendment and Reply filed March 5, 2005.

Declaration under 37 C.F.R. 1.132 by Dr. Thomas Dag Horn, dated 8/18/2005. Submitted with the Appeal Brief filed August 24, 2005.

APPENDIX III

Cases Cited

In re Tanczyn, 347 F.2d 830, 146 U.S.P.Q. 298 (C.C.P.A. 1965).

In re Hostettler, 356 F.2d 562 (C.C.P.A. 1966).

In re Stryker, 435 F.2d 1340 (C.C.P.A. 1971).

In re Spiller ,500 F.2d 1170 (C.C.P.A. 1974)).

Ex parte Levy, 17 U.S.P.Q.2d 1461 (Bd. Pat. App. & Inter. 1990)

In re Rijckaert, 9 F3d 1531 (Fed. Cir. 1993).

In re Best, 562 F.2d 1252 (C.C.P.A. 1977)

Ex parte Gray, 10 U.S.P.Q.2d 1922 (Bd. Pat. App. Int. 1989).

In re Fessmann, 489 F.2d 742, 180 U.S.P.Q. 324 (C.C.P.A. 1974).

In re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Package Insert Text – Candin®

DESCRIPTION

Candida albicans Skin Test Antigen for Cellular Hypersensitivity (CANDIN®) is a clear, colorless, sterile solution with a pH of 8.0 - 8.5. The antigen should be administered intradermally according to the directions included under DOSAGE AND ADMINISTRATION of this package insert.

CANDIN® is made from the culture filtrate and cells of two strains of Candida albicans. The fungi are propagated in a chemically defined medium consisting of inorganic salts, biotin and sucrose. Lyophilized source material is extracted with a solution of 0.25% NaCl, 0.125% NaHCO3 and 50% v/v glycerol. The concentrated extract is diluted with a solution of 0.5% NaCl, 0.25% NaHCO3, 0.03% Albumin (human) USP, 8 ppm polysorbate 80 and 0.4% phenol.

The skin test strength of CANDIN® has been determined from dose-response studies in healthy adults. The product is intended to elicit an induration response ≥5 mm in immunologically competent persons with cellular hypersensitivity to the antigen.

The potency of CANDIN® is measured by DTH skin tests in humans. The procedure involves concurrent (side-by-side) testing of production lots with an Internal Reference (IR), using sensitive adults who have been previously screened and qualified to serve as test subjects. The induration response at 48 hours elicited by 0.1 mL of a production lot is measured and compared to the response elicited by 0.1 mL of the IR. The test is satisfactory if the potency of the production lot does not differ more than ±20% from the potency of the IR, when analyzed by the paired t-test (two-tailed) at a p value of ≤ .05.

The potency of the IR is monitored by DTH skin testing. Persons included in the potency assay are qualified as test subjects by receiving four skin tests with the IR from which a mean induration response (mm) is calculated. Current skin tests with the IR must show that the potency of the IR has not changed more than ± 20% from the mean qualifying response in the same test subjects, when analyzed by the paired t-test (two tailed) at a p value of 0.05. The required induration response at 48 hours to the IR is 15 mm ± 20%.

CLINICAL PHARMACOLOGY

Cellular or delayed-type hypersensitivity (DTH) can be assessed by intracutaneous testing with bacterial, viral and fungal antigens to which most healthy persons are sensitized. A positive skin test denotes prior antigenic exposure, T-cell competency and an intact inflammatory response (1,2). The reaction usually peaks 48 hours after antigen is introduced into the skin and is manifest as induration at the test site.

Recall antigens may be useful in evaluating delayed-type hypersensitivity by eliciting positive induration reactions 48 to 72 hours after intracutaneous administration. Except for mumps skin test antigen, most commonly used recall antigens were developed for other purposes, and the size of the reaction elicited may not be directly related to cellular immunity because of variability in antigen source and dose and skin test administration and measurement techniques. Useful antigens are those which elicit a reaction size ≥5 mm in more than 50% of normal individuals. The

combination of results from skin testing with more than one antigen should result in detection of DTH in at least 95% of normal subjects (2).

The inflammatory response associated with the DTH reaction is characterized by an infiltration of lymphocytes and macrophages at the site of antigen deposition. Specific cell types that appear to play a major role in the DTH response include CD4+ and CD8+ T lymphocytes which leave the recirculating lymphocyte pool in response to exogenous antigen (3). Both CD4+ and CD8+ lymphocytes have been recovered from DTH reactions elicited by Candida antigen (4).

In the literature, the incidence of DTH reactions to unstandardized Candida antigens has been reported to vary from 52 - 85 %, depending upon the strength of the antigen and the mm induration required for a positive test (5,6,7,8,9).

Published studies have reported that antigens of Candida albicans are useful in the assessment of diminished cellular immunity in persons infected with human immunodeficiency virus (10,11). Responses to DTH antigens have been reported to have prognostic value in patients with cancer (5).

Table1. Cellular hypersensitivity response to CANDIN® in healthy adults (15).

Study	Gender	N	Age range (years)	Number reactions 5 mm at 48 hours	Response overall
Study 1 (a)					
	Male	16	25-83	14	89%
	Female	2	61-69	2	
Study 2					
	Male	20	23-63	13	60%
	Female	15	28-62	8	

(a) Control group in Table 2.

RESPONSE TO CANDIN® in Healthy Adults (Table1): In one group of 18 subjects, 14 (78%) of the individuals reacted to CANDIN® with an induration response of ≥5 mm at 48 hours. In a second study of 35 subjects, 21 (60%) had induration reactions ≥5 mm at 48 hours. In this study, 65% of males tested positive compared to 53% of females; the mean induration in responding males was 12.8 mm and in responding females was 13.0 mm. When subjects in these studies were tested with two reagents, CANDIN® and Mumps Skin Test Antigen, 92% were positive to at least one antigen, a higher response rate than to either antigen used alone (15).

Table 2. Cellular hypersensitivity response to CANDIN® in adults with AIDS, adults with HIV infection (no-AIDS-indicator conditions) and adult control subjects (15).

Group	Classification*	N	Zidovudineuse	CD4T-cell	Mean induration	N≥5	%	

				count	(mm)			
				Range	Mean			
AIDS	A3, B3, C	32	14	4-483	145	3.35 (a)	9	28 (b)
HIV Pos.	A1, A2, B1, B2	28	13	201-1085	455	5.67	15	64
Control	-	18	0	554-1876	869	8.03	14	78

(reference 12) (a) p= 0.01compared to Control.(b) p< 0.01compared to Control.

RESPONSE TO CANDIN® in Adults with HIV Infection: In one study (Table 2). the skin test responses of adults with HIV infection were compared to those of healthy control subjects (age range AIDS 22-65, HIV positive 20 - 45, Controls 25 - 69). When HIV infected subjects were classified by the CDC's 1993 revised classification system for H.I.V infection (12), a significant difference was found between AIDS patients and normal controls in both mean induration (p = 0.01) and proportion with ≥5 mm response (p< 0.01). The responses in HIV-infected patients (without AIDS-indicating conditions or AIDS-indicating CD4 T-cell counts) were less than in normal subjects, but the differences were not statistically significant.

In a second study involving 20 male patients (age range 26 - 57) diagnosed with AIDS based on clinical criteria only, one subject responded to CANDIN®. In the same study 65% of the male control subjects had DTH reactions ≥ 5 mm to CANDIN® (Table 1, Study 2). The mean induration response at 48 hours for control subjects was 8.33 mm compared to 1.78 mm for the AIDS subject. AIDS vs. control p-values were < 0.01 mean induration and <0.01 induration 5 mm.

Because HIV infection can modify the DTH response to tuberculin, it is advisable to skin test HIV-infected patients at high risk of tuberculosis with antigens in addition to tuberculin (16). In a published study of DTH anergy, 179 subjects (334 males and 145 females) infected with HIV and being screened for tuberculosis were skin tested with several additional antigens, including CANDIN® supplied under IND to the investigators. Only 12% reacted to tuberculin (≥5 mm), 57% reacted to CANDIN® (≥3 mm) and 60% reacted to either tuberculin or CANDIN® or both. In this study, a 3 mm induration response to CANDIN® was considered positive. The authors concluded that in HIV-infected subjects, testing with other DTH antigens increases the accuracy of interpretation of negative tuberculin reactions.

Table 3. Cellular hypersensitivity response to CANDIN® in adults with cancer (15).

	N	Age Range	Number reactions 5 mm at 48 hours	Response
Study 1	18	52-75	5	28%
Study 2	20	47-81	0	0%

In one study of 18 patients with lung cancer, CANDIN® elicited a positive induration response in five patients (28%). In a second series of 20 patients with metastatic cancer, no reactions ≥5 mm were observed (Table 3).

INDICATIONS

CANDIN® is indicated for use as a recall antigen for detecting DTH by intracutaneous (intradermal) testing. The product may be useful in evaluating the cellular immune response in patients suspected of having reduced cellular hypersensitivity. Because some persons with normal cellular immunity are not hypersensitive to Candida, a response rate less than 100% to the antigen is to be expected in normal individuals. Therefore, the concurrent use of other licensed DTH skin test antigens is recommended. The product should not be used to diagnose or treat Type 1 allergy to Candida albicans.

DOSAGE AND ADMINISTRATION

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If particles or discoloration are observed, the product should not be used and it should be discarded.

CANDIN® should be administered intradermally on the volar surface of the forearm or on the outer aspect of the upper arm. The test dose is 0.1 mL. The skin should be cleansed with 70% alcohol before applying the skin test. The intradermal injection must be given as superficially as possible causing a distinct, sharply defined bleb. An unreliable reaction may result if the product is injected subcutaneously. A positive DTH reaction to CANDIN® consists of induration ≥5 mm.

The time required for the induration response to reach maximum intensity varies with the individual. The reaction usually begins within 24 hours and peaks between 24 and 48 hours. The skin test should be read at 48 hours by visually inspecting the test site and palpating the indurated area. Measurements should be made across two diameters as shown in the figure below. The mean of the longest and midpoint orthogonal diameters of the indurated area should be reported as the DTH response. For example, a reaction that is 10 mm (longest diameter) by 8 mm (midpoint orthogonal diameter) has a sum of 18 mm and a mean of 9 mm. The DTH response is therefore 9 mm

ADVERSE REACTIONS

Local reactions to CANDIN® have included swelling, pruritus and vesiculation. Reactions involving necrosis and ulceration have not been observed, but such reactions are theoretically possible and might occur in persons with exquisite cellular hypersensitivity to the antigen. Local reactions may be treated with a cold compress and topical steroids. Severe local reactions may require additional measures as appropriate.

In a published study (13) of 479 HIV positive adults tested with CANDIN®, adverse local reactions were observed in six subjects as follows: pruritus (three), swelling at the test site (one), vesiculation (one)l and vesiculation with weeping edema (one). Pruritus and swelling cleared within 48 hours; vesiculation with edema required approximately 1 week to resolve (15).

In two studies involving 171 persons discussed under CLINICAL PHARMACOLOGY in Tables 1, 2, 3, and text, one adverse reaction was observed. This reaction consisted of induration 22 x 55 mm at 48 hours which resolved within 1 week (15).

Testing of CANDIN® for consistency of potency is performed in healthy human subjects who are known to be skin-test positive to the antigen. In 58 subjects tested to-date, there have been no cases of Type 1 allergy manifested as either generalized or adverse local reactions. One subject had induration with a central vesicle which subsided within a few days (15).

Severe local reactions, including rash, vesiculation, bullae, dermal exfoliation and cellulitis, have been reported to MedWatch for unstandardized allergenic extracts of Candida albicans used for anergy testing (17).

Systemic reactions to CANDIN® have not been observed. However, all foreign antigens have the remote possibility of causing Type I anaphylaxis (14) and even death when injected intradermally. Systemic reactions usually occur within 30 minutes after the injection of antigen and may include the following symptoms: sneezing, coughing, itching, shortness of breath, abdominal cramps, vomiting, diarrhea, tachycardia, hypotension and respiratory failure in severe cases. Systemic allergic reactions including anaphylaxis must be immediately treated with Epinephrine HCl 1:1,000. Additional measures may be required, depending upon the severity of the reaction.

IMMEDIATE HYPERSENSITIVITY reactions to CANDIN® occur in some individuals. These reactions are characterized by the presence of an edematous hive surrounded by a zone of erythema. They occur approximately 15-20 minutes after the intradermal injection of the antigen. The size of the immediate reaction varies depending upon the sensitivity of the individual. Immediate hypersensitivity reactors were observed in the control and HIV-infected (AIDS and HIV positive) subjects reported in Table 2 as follows: HIV-infected subjects (20% with erythema of 10-21 mm in diameter; 13% with erythema of 5-9 mm). Control subjects (22% with erythema of 10-15 mm; 5% with erythema of 3-5 mm). Cancer subjects (CLINICAL PHARMACOLOGY: Table 3, Study 1), 17% with erythema of 10-24 mm and 11% with erythema of 6-9 mm.

DRUG INTERACTIONS

Pharmacologic doses of corticosteroids may variably suppress the DTH skin test response after two weeks of therapy. The mechanism of suppression is believed to involve a decrease in monocytes and lymphocytes, particularly T-cells. The skin test response usually returns to the pretreatment level within several weeks after steroid therapy is discontinued (1).

WARNINGS

As has been observed with other, unstandardized, antigens used for DTH skin testing (14), it is possible that some patients may have exquisite immediate hypersensitivity to CANDIN®. In persons with bleeding tendency, bruising and non specific induration may occur due to the trauma of the skin test.

PRECAUTIONS

General

Physicians using this product must have available the facilities and medications necessary to treat all potential local and systemic side effects that can occur from the injection of an antigenic substance. Epinephrine (1:1,000) must be immediately available. The patient, or parent or guardian, should be questioned about previous reactions to this product or a similar product.

The antigen must be injected intradermally as superficially as possible, causing a distinct, sharply defined bleb at the skin test site. An unreliable reaction may result if the product is injected subcutaneously. It must not be given intravenously; care should be taken to avoid injection into a blood vessel.

A separate sterile syringe and needle should be used for each patient to prevent transmission of infectious agents. Needles should be disposed of properly and should not be recapped.

Delayed or cellular hypersensitivity reactions can be diminished or completely suppressed if the person has received corticosteroids (see DRUG INTERACTIONS).

Patient Information

See PATIENT INFORMATION section.

Drug Interactions

See DRUG INTERACTIONS section.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long term studies in animals have not been conducted with CANDIN® to determine its potential for carcinogenicity, mutagenicity or impairment of fertility.

Pregnancy

Category C: Animal reproduction studies have not been conducted with CANDIN®. It is also not known whether CANDIN® can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. CANDIN® should be given to pregnant women only if clearly needed.

Nursing Mothers

It is not known whether CANDIN® is excreted in human milk. Because drugs may be excreted in human milk, caution should be exercised when this product is administered to a nursing woman.

Pediatric Use

The safety and effectiveness of intradermally administered CANDIN® have not been established in children.

Geriatric Use

CANDIN® has not been adequately studied in geriatric patients. However, the DTH response to CANDIN® may be diminished in geriatric patients, since the aging process is known to alter cell mediated immunity (1).

OVERDOSAGE

No information provided.

CONTRAINDICATIONS

CANDIN® should not be used after a previous unacceptable adverse reaction to this antigen or to a similar product, i.e., extreme hypersensitivity/allergy

PATIENT INFORMATION

Local reactions to CANDIN® can include redness, swelling, pruritus, excoriation and discoloration of the skin. These reactions usually subside within hours or days after administration of the skin test. In some patients, skin discoloration may persist for several weeks. Progression of the DTH reaction to vesiculation, necrosis and ulceration are possible. Patients should be informed that all foreign antigens have the remote possibility of causing Type I anaphylactic reactions that may require the administration of epinephrine and other drugs or emergency procedures and may be life threatening in some cases. Patients should report any serious adverse reactions to their health care provider

HOW SUPPLIED

CANDIN® is supplied in a 1 mL multidose vial containing ten 0.1 mL doses.

STORAGE

Store between 2 - 8°C. Do not freeze.

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The Physiology of Immunity

Edited by

James A. Marsh, Ph.D.
Department of Microbiology and Immunology
Cornell University
Ithaca, New York

Marion D. Kendall, Ph.D., D.Sc.
Thymus Laboratory
The Babraham Institute
Cambridge, United Kingdom

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CHAPTER 1

Histophysiology of the Lymphoid System: The Thymus and T Cells

Paul Nieuwenhuis

CONTENTS

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I. INTRODUCTION: IMMUNITY, THE IMMUNE SYSTEM, AND THE CNS

In ancient times, Roman citizens could be granted "immunity", i.e., freedom from obligations to the state ("immunitas", from "munus": task, job, obligation [to the republic, "res publica"]). With time, the word also took on the meaning of being free from contamination, clean, and pure. Thus the word "immunis" (immune) also came to refer to freedom from disease, especially those of an infectious nature.

Present-day diplomats, etc. can claim immunity so as not to become involved in, e.g., a process of prosecution. For ordinary people, however, immunity mostly is considered to be a state of increased resistance or proof to all sorts of infectious diseases resulting either from contracting a particular disease and surviving it or from a successful vaccination program.

The human organism is constantly threatened by the possibility of an invasion of various kinds of microorganisms like bacteria, viruses, or parasites which, when violating the integrity of the body, might upset the delicate balance of the "milieu intérieur". To combat these infections, during evolution a highly sophisticated immune system was developed specifically dedicated to eliminate any invading microorganism (antigens).

Basically, this immune system operates at two levels and accordingly can be split into (1) the innate (or nonspecific and nonadaptive) immune system, and (2) the cognate (or specific and adaptive) immune system.[1]

A *first* essential difference between these two is that the innate immune system can function everywhere in the body in response to an invading microorganism as the cells involved in this system can be directed towards the site of invasion, whereas for the cognate immune system to function properly, specially structured microenvironments (peripheral lymphoid organs) are needed where the cells involved in this system can meet the antigen, which is specifically transported from its *porte d'entrée* to these microenvironments and presented to potentially responsive cells.

A *second* difference is that the cells involved in the innate immune system use rather primitive recognition systems unable to discriminate between various types of microorganisms. Upon recognition, the microorganism is phagocytized and, by the use of intracellular lytic enzymes, killed and digested. Typical examples of these cells are polymorphonuclear granulocytes and monocytes (macrophages). On the other hand, the cells in the cognate immune system carry highly diverse and specific receptors by which they can recognize antigenic epitopes of the invading microorganism when properly presented to them. As a result of this recognition, which involves only a fraction of the cells present, responding cells undergo a process of amplification by cell proliferation thereby increasing the number of effector cells by many orders of magnitude. Cells in the cognate immune system have become known as T cells and B cells.

A *third* difference is that upon elimination of the intruder by the innate immune system the organism as such has not changed and only the status quo has been restored. It has not learned from this encounter. However, in the cognate immune system, as a result of the highly specific recognition of the antigen by the responding cells, specific antibodies and/or sensitized cells are formed that assist in the elimination of the antigen and confer a state of immunity upon the organism, making a renewed invasion by the same type of microorganism even less likely to be successful. Moreover, as part of the response memory cells are formed, which upon secondary invasion are capable of mounting a more vigorous and even more effective attack on the invader. Due to the action of the cognate immune system the organism

has changed (has adapted) and a new balance has been reached where it is better equipped to deal with renewed invasion. Moreover, it has learned from this encounter, this knowledge being stored away in the memory cells, which can be called upon as long as these memory cells live.

Evidently the two systems are complementary to each other. As a result of the action of the cells involved in the first line of defense (local inflammatory response) breakdown products of the invading microorganism are taken up by (nonlymphoid) antigen presenting cells (APC) and in a cell-bound form are transported through afferent lymphatics to the nearest draining lymph node. Here, naive but highly specific T and B cells, which have specifically migrated to these lymph nodes, interact with the presented antigen giving rise to effector molecules like antibodies (produced by plasma cells which originate from activated B cells) and effector cells like cytotoxic T cells. By way of the efferent lymphatics these cells and molecules reach the systemic circulation, from where they may extravasate at the site of infection to more effectively eliminate the source of the infection.

For some time now it has become known that this interplay between the cells involved is highly regulated by special mediator molecules or cytokines which in a paracrine way stimulate and affect cells and structures (like blood vessels) at the site of inflammation as well as in the draining lymph node (intrinsic regulation). However, since the late 1970s it has also become apparent that the whole of the immune system is also under neuroendocrine control, perhaps even involving psychosocial factors mediated by the neuroendocrine system (extrinsic regulation).[2] This holds not only for those instances where the immune system is actually challenged and typical responses may be influenced by central nervous system (CNS) activity, but also in the pre-immune state by regulating growth and differentiation and the normal physiology of cells (like migration) and structures involved.

A well-known example of this is the observation that, with age, the thymus, at least to a large extent, becomes atrophic. This largely depends on the production of sex hormones, as castration even at an advanced age can prevent or restore thymus (dys)function. Also, in the chicken, atrophy of the bursa of Fabricius (from which in birds B cells derive) occurs at sexual maturity, and castration before this time can prevent this involution, resulting in continued growth and function of the bursa.

However, CNS activity is not only mediated by the hypothalamo-pituitary (H-P) axis and the endocrine organs, depending on trophic hormones produced by the latter (like gonadotrophic hormone, thyrotrophic hormone, adrenocorticotrophic hormone), and hormones produced by the pituitary itself (like growth hormone and prolactin). It is also mediated by direct neuronal control, especially by the sympathetic branch of the autonomic nervous system, e.g., by regulating blood flow through organs and tissues involved in immune responses.

Most exciting, however, were observations from which it became apparent that certain substances produced during a local inflammatory response also affected the CNS, thereby eliciting CNS activity and resulting in the release of hormones that eventually would down-regulate the immune response. A typical example of this is interleukin-1 (IL-1) which, apart from local effects at the site of inflammation, also stimulates the H-P axis to produce and release corticotrophin-releasing hormone (CRH) acting upon the adenohypophysis to release ACTH. This in turn stimulates the adrenal cortex to secrete glucocorticoids, which are known to have an immunosuppressive effect.[3] Since these early observations, a plethora of data have become available showing that mediator molecules used in the intrinsic regulation of the immune system also affect the CNS, and neuroendocrine transmitters may even be produced by the lymphoid system itself. As such, there is a constant stream of information from the immune system to the CNS, which in turn may respond and further assist in regulating immune processes. Thus, to some people the immune system is considered as an extension of the abiltiy of the CNS to recognize stimuli that the CNS itself would not be capable of recognizing. In addition to the usual five senses such as smell, touch, pain, hearing, and sight,

the immune system has been considered to be the organism's "sixth sense" to monitor its boundaries for the possible invasion of unwanted intruders.[2]

The awareness of a complex integration between the neuroendocrine and the immune systems reflects the renewed attention for the unraveling of immune phenomena at the level of the whole organism by studying *in vivo* immunology. It is only by this holistic approach that factors governing health and disease can be identified in their proper context and perhaps eventually be used in the cure and prevention of immune-associated diseases.

II. THE COGNATE IMMUNE SYSTEM: DELINEATION OF T AND B CELL SYSTEMS

Historically, until the first half of this century knowledge of immunity to infectious diseases developed completely separate from knowledge of the lymphoid system, which we now know as the cognate immune system (CIS). During the period ranging from the earliest recognition of "Lymph-zellen" (lymph-o-cytes) in the beginning of the nineteenth century to the present-day knowledge of the CIS, two lymphoid organs, which eventually were to play a major role in the delineation of the T and B cell systems, viz., the thymus and the Bursa of Fabricius, were considered to have endocrine functions. It might be worthwhile to dwell for a while on how this knowledge came about.

A. THE LYMPHOCYTE IN AN HISTORICAL PERSPECTIVE

Initially, lymphocytes were discovered in the lymph (chyle) draining the intestinal mucosa. A major question at that time was whether these cells originated from cells in the mesenteric lymph node ("omnis cellula e cellula") or whether these cells were merely fusion products of the fat droplets (chylomicrons) that give the chyle its white milky appearance ("generatio spontanea": the generation of living cells from dead material). In an ingenious experiment involving feeding rabbits a low-fat-containing diet, Bruecke (1854) observed that despite the absence of fat droplets in the chyle the number of lymphocytes had not decreased, and he concluded: "Es ist gewiss und unzweifelhaft dass die Lymphkörperchen in die Lymphdrüsen gebildet werden, und zwar nicht aus Keimen, welche der Chylusstrom in dieselben hineinbringt, sondern aus solchen, welche sich auf dem Drüsengewebe, als auf ihren mütterlichen Boden, entwickeln".[4] (See end of chapter for English translations.)

Later these cells were also identified in what we now know as lymphoid organs like Peyer's patches ("Peyerischen Drüsen"), lymph nodes, spleen, and blood. To this list the thymus was added by His (1861) and the concept of a system of lymphatic tissues developed.[5] In this context it is of interest to note that in ancient Greece the thymus was considered to house the soul ("thumos" = life force, mind, thought, character) and that by its special position in the mediastinum anterior, i.e., overlying the heart, it could influence this organ. Presumably, the herb thyme (*Thymus vulgaris*) derives its name from the thymus (and not vice versa!) due to the resemblance of its leaves to the shape of the two thymic lobes.

Meanwhile, it had been noticed (Kölliker, 1859) that the efferent lymph contained many more lymphocytes than the afferent lymph, which again raised the question of their possible origin.[6] Flemming (1885) considered two possibilities: "Entweder sie erfolgt durch Theilungen der Zellen, die in den Maschen des Retikulums der Knoten und Strängen lagern; oder sie geschieht in der Art das aus den Blutgefässen dieser Knoten und Strängen fortdauerend oder schubweise Leukozyten auswandern, so dass also einer Art kontinuierlichen Kreislaufs dieser Elemente aus dem Blut in die Lymphe und mit dieser wieder ins Blut statt finden würde".[7] Careful histological observation by Flemming disclosed high mitotic activity in the lightly staining centers of the secondary nodules (follicles) in the outer cortex of the investigated lymph nodes. He named these areas of high mitotic activity "Keimzentren" (germinal

centers) and he concluded: "Die Lymphknoten und die 'Darmfollikel' sind Brutstätten der Neubildung von Lymphozyten auf dem Wege indirecten Theilungs." However, by finding this high mitotic activity, Flemming unfortunately overlooked the fact that his two hypotheses were not mutually exclusive; he thus did not pursue the other possibility and thereby missed the concept of recirculation of lymphocytes, as it much later (1958) was formulated by Gowans, although he was the first to speculate about this possibility.[8]

When investigating the thymus, mitotic figures, surprisingly enough, were not found in the lighter-staining central parts (medulla) but in the darker-staining surrounding rim of lymphocytes (cortex). Nevertheless, Flemming stated: "(es wird) kaum zu bezweifeln sein, dass es (i.e., the thymus, Nieuwenhuis) während der Periode seiner vollen Ausbildung der Neulieferung von Lymphzellen ebenso dient, wie später die Lymphdrüsen und lymphoide Organe."[7]

Several years later this postulated thymic origin of lymphocytes was reinvestigated by Beard, who reported these investigations in two papers: "The Development and the Probable Function of the Thymus" (Beard, 1894) and "The Source of Leukocytes and the True Function of the Thymus" (Beard, 1900).[9,10] From his investigations into the embryonic development of the thymus, where he thought the first lymphocytes in the body to arise (albeit from its epithelial cells!), he concluded: "it must therefore be held, and in my humble opinion the contrary is impossible of proof, that the thymus is the parent-source of all the lymphoid structures in the body." Finally, he suggested: "that, if a lymphoid organ arises later elsewhere, it will always be impossible to prove, that it did not take its origin from some of the leukocytes, or their progeny, which originally came from the thymus", thus linking the lymph node origin of lymphocytes to their postulated thymic origin.[10]

In 1909, Hammar published a paper entitled: "Fünfzig Jahre Thymusforschung" in which he reviewed thymic research up to that moment, even going back as far as 1659 to a description of the thymus by Wharton, who because of the lobular structure of the thymus compared it to the pancreas, thinking it was a composite gland — a view that stuck for a long period of time, even though an excretory duct was never found[11]. Many investigators, however, observed some sort of cavity or cavities within the thymus filled with a white limpid fluid. Others, however, claimed that these cavities (presumably representing what we would now call the medulla) were artifacts induced during the preparation of the "gland" for observation, as during this preparation a white fluid could be pressed from the respective lobules. Hewson, in 1777, as quoted by Hammar, was the first to study this "white fluid" microscopically, finding "numberless small particles precisely corresponding with those found in the fluid of the lymphatic vessels passing from the thymus and with those formed in the fluid of the lymphatic glands." It should not be forgotten that in those days proper fixation of tissues as performed today was nonexistent, tissues sometimes being cooked before being hardened by immersion in alcohol.

A most puzzling fact in determining what kind of organ or tissue the thymus was, was the observation that during embryonic development the earliest thymic "anlage" is definitely epithelial in origin, making a glandular structure not unlikely. In 1921, Hammar, summing up the results of his own studies ominously enough published in *Endocrinology* stated: "All these facts contain as many confirmations...of the fact that the thymus is not or does not consist of lymphoid tissue" and: " the thymus is an epithelial organ, infiltrated with lymphocytes."[12] At that time most thymus research was centered around a possible function not within the lymphoid system but within the field of endocrine regulation, even though the two classical experiments to show endocrine function consistently failed to provide any support for that view. Ideally, for an organ to have an endocrine function two criteria should be met: (1) elimination of that organ should lead to some sort of metabolic disturbance which (2) could be restored to normal by injecting an extract of the organ under study. In those days, thymectomy experiments consistently failed to induce any observable alterations whatsoever and thymic extracts were not found to have any effect.

B. RECIRCULATING LYMPHOCYTES

While the origin of lymphocytes, as they occur in peripheral lymphoid organs, thoracic duct lymph, and blood was difficult to establish, so were scientists by the middle of this century hard put to ascribe a functional role to these cells. In 1956, decades of intensive research as to the enigmatic character of the lymphocyte were comprehensively reviewed by Yoffey and Courtice[13] as follows: "The lymphocyte is a somewhat inconspicuous cell, with no particularly striking functional or morphological characteristics.... Comparing it with other cells one thinks of the lymphocyte in negative terms, defining it rather by the absence of characteristics which other white cells possess, than by positive attributes of its own"[13]. And, quoting Lewis (1932) he stated: "Its fate...is the subject of religious beliefs."

One of the mysteries concerning lymphocyte physiology was the observation that although large numbers of lymphocytes are poured into the bloodstream daily by way of the thoracic duct, sufficient to replace the number of blood-borne lymphocytes several times, their level remained constant. Suggestions as to the fate of these cells ranged from emigration along the gastrointestinal tract into its lumen, where they would perform their final task (Bunting and Huston, 1921), to passage into the bone marrow to serve as stem cells for the other blood cells (Jordan, 1939; Yoffey and Darnell, 1944).[14,15,16] However, no evidence could be obtained to support these views. In 1936, Sjøvall suggested the emigration of blood-borne lymphocytes into peripheral connective tissues and a return of these cells by way of the lymphatics back to the bloodstream.[17] However, Yoffey and Drinker (1939) calculated that in this way only 1 out of 30 lymphocytes in the efferent lymph could be accounted for.[18] A beginning of a solution to this conundrum came from experiments by Farr (1951) using autologous transfusion of labeled lymphocytes.[19] Upon i.v. administration these cells were found to be rapidly removed from the circulation and some of these cells were found to have entered peripheral lymphatic tissues. Referring to earlier observations by Schulze (1925) on the presence of lymphocytes in the wall of postcapillary venules in lymph nodes, Farr suggested that instead of leaving the lymph node to enter the bloodstream it might be that these cells were leaving the circulation to enter the lymph node.[19,20]

Final proof came from experiments by Gowans et al. in the 1950s who showed that i.v. infused thoracic duct lymphocytes subsequently would reappear in the thoracic duct lymph, suggesting a recirculation of these cells presumably through the lymph nodes, which eventually was demonstrated by means of autoradiography. Labeled lymphocytes, within 15 min after reinfusion, could be identified in the wall and the immediate surroundings of the postcapillary venules in the deep cortex of the lymph nodes.[21,22]

This concept of recirculation (and redistribution!) of lymphocytes through peripheral lymph organs, however, still did not give a clue to the two major questions concerning lymphocyte physiology, viz: (1) what is the origin of these cells and (2) what is their fate?

C. NEWER CONCEPT OF T AND B CELLS

In 1604, a small pear-shaped diverticulum extending from the dorsal side of the cloaca in chickens was described for the first time by Fabricius. Later this structure became known as the bursa of Fabricius. It seems that Fabricius believed that this structure was only present in females and served as a semen reservoir. However, later studies showed that in either sex the bursa disappeared at sexual maturity, which was interpreted by some authors as indicating that the bursa played a role in the attainment of sexual maturity. To substantiate this view, bursectomy experiments were performed at several intervals posthatching to see if sexual maturation could thus be advanced. However, this appeared not to be the case. On the other hand, castration before sexual maturity prevented bursal involution whereas injections with testosterone propionate (TP) advanced bursal involution. At that time the lymphoid nature of

the bursa had not yet been established although on morphological grounds some authors had compared it with the thymus (for review see Reference 23 and Chapter 10, this volume).

Up to the summer of 1954 bursectomy experiments had failed to reveal a specific function for the bursa. Then, a chance observation was made during a class demonstration on antibody formation; several of the *Salmonella typhimurium* O antigen-injected chickens failed to produce antibodies. When wing-band numbers were checked it appeared that these nonresponders had previously been bursectomized.[23,24] Further experimentation, also including chemical (or hormonal) bursectomy by the injection of TP *in ovo*, clearly linked (the development of) antibody-forming capacity to the presence of the bursa of Fabricius and presumably to cells derived from it, later to be known as B (= bursa-derived) cells.[25] In the spleens of chickens bursectomized immediately posthatching, a particular set of lymphoid cells surrounding the Schweigger-Seidel sheaths around the terminal arterioles was lacking, as were germinal centers. Sera from these animals showed virtual absence of the gamma globulin fraction, known to contain antibody activity (agammaglobulinemia).

That lymphoid cells in peripheral lymphoid organs like lymph nodes and spleen may be instrumental in immune responsiveness had already been anticipated by the work of McMaster and Hudack (1935), who showed that after s.c. injection of an antigen specific antibodies to that antigen could be demonstrated several days later in the efferent lymph of the draining lymph node.[26] Fagraeus (1948), and later Keuning et al., gave detailed descriptions of the changes in both spleen and lymph nodes after i.v. or s.c. administration of an antigen. Histologically, large numbers of large pyroninophilic cells, presumably developing from small lymphoid cells and subsequently differentiating into immature and mature plasma cells, were observed as morphologically representing the immune response leading to antibody formation and humoral immunity.[27,28] This histological reaction accordingly was called "plasmacellular reaction" (PCR).[27]

However, following other kinds of antigenic stimulation, e.g., the cutaneous grafting of a piece of foreign skin (allograft) or the cutaneous application of a chemical sensitizer, the resulting "immunity" was found to be associated with the occurrence within the blood of specifically "sensitized" cells and not with the presence of antibodies.[29] Whereas the plasmacellular reaction in lymph nodes predominantly occurred in the outer cortex containing the follicular structures, this cellular immune response, also involving the transition to large pyroninophilic cells which, however, further developed back to small lymphoid cells, was mainly located in the deep cortical area.[30] Thus, histologically, two types of immune responses could be observed, depending upon the eliciting antigen, localized in distinctly different areas of a peripheral lymphoid organ and giving rise to cell-mediated (cellular) or antibody-mediated (humoral) immunity.

Surprisingly, upon antigen administration either s.c., i.v., or i.p., changes as described above were never observed in the thymus. In addition, repeated experiments involving thymectomy, but now with regard to possible changes in immune reactivity, consistently proved negative even though a general fall in the lymphocyte population of the blood, thoracic duct lymph and peripheral lymphoid organs could be observed. These results made MacLean et al. (1957) conclude: "The thymus does not participate in the control of immune responses".[31] Their experiments, however, were performed in young adult rabbits. Thymectomy at or soon after birth, by contrast, was found to be associated with more serious defects. Numbers of lymphocytes in blood and lymphoid tissues were severely decreased about 5 to 6 weeks after birth in neonatally thymectomized mice.[32] In addition these animals showed significant impairment of immunological capacity as witnessed by failure to reject foreign grafts of skin when grafted several weeks after thymectomy. In some strains of mice, neonatal thymectomy was associated several months later with development of the"wasting or runts' disease" characterized by general impairment of growth, weight loss, lethargy, ruffled fur, hunched back, and eventual death.[32] Later, Bianchi et al. (1971) showed this condition to be associated

with degranulation of acidophilic cells of the anterior pituitary and loss of growth hormone and prolactin production, suggesting a role for the thymus, either directly or indirectly, in the production of these hormones![33]

The fact that foreign skin grafts were accepted when grafted well before the onset of this wasting disease indicates that impairment of allograft rejection, and thus cellular immunity, did not result from whatever might cause this disease. Rather, this was likely a direct effect of the loss of a particular type of lymphocyte apparently derived from the thymus. In 1962, Szenberg and Warner observed in chickens that hormonal bursectomy in some animals also led to partial or even complete atrophy of the thymus.[25] When these animals were tested with a variety of antigenic challenges it turned out that there were at least two basic levels of immune response: (1) the first level associated with the production of circulating antibody (humoral immunity) and dependent upon the presence of the bursa as the primary lymphoid organ, and (2) a second level of immune response concerned with recognition of histocompatibility antigens like foreign skin grafts (cellular immunity) and dependent upon the presence of the thymus.

Mammals, however, lack a bursa of Fabricius and since, in the mouse, neonatal thymectomy not only impaired cellular immunity but also antibody production to some antigens, the mammalian thymus was at first believed to fulfil the function of both the thymus and bursa of birds.[32] Histologically, however, neonatal thymectomy in mice and rats had been found predominantly to affect (by severe depletion) those areas in peripheral lymphoid organs (e.g., in the lymph node: the deep cortical area) associated with immune responses leading to cellular immunity rather than those areas where the plasmacellular reaction was believed to start, i.e., the outer cortex with follicles and germinal centers.[34,35] "It is concluded that small lymphocytes of the spleen and lymph nodes may come, in large part, directly from the thymus and are not derived from medium and large lymphocytes of the germinal center. It is suggested that there may be a second population of small lymphocytes whose function is unrelated to the thymus lymphocytes."[34]

These observations led some investigators on a frantic search for a postulated "bursa equivalent" in mammals and for a while the so-called "gut-associated lymphoid tissue" (GALT) like tonsils, Peyer's patches, and appendix were considered the prime candidates for this function.[36] In the meantime, the effect of neonatal thymectomy could be mimicked by thymectomy of adult mice or rats and even rabbits when followed by whole-body X-irradiation (to wipe out remaining cells) and some form of bone marrow reconstitution to allow the animals to survive.[37] Again, histologically, certain areas like the periarteriolar lymphocyte sheath in the spleen and the deep cortex in lymph nodes were found completely devoid of any lymphoid cells, whereas follicular structures were found to be restored to near normal levels coinciding with the acceptance of foreign skin grafts and the restoration, to some antigens at least, of antibody-forming potential. Final clues came from experiments by Mitchell and Miller (1968) using genetically marked cells which could be detected by the use of specific antisera in bone marrow reconstituted radiation chimeras and they concluded "that the precursors of the hemolysin forming cells are not derived from ... thymus ... lymphocytes but from bone marrow."[38] This was the first unequivocal proof that thymus-derived cells did not become antibody-formers but were required in many antibody responses to focus antigen onto potential antibody forming cells and *help* them produce antibody.[39] So the source for antibody-forming cell precursors in mammals seemed to be the bone marrow, and as this tissue also started with "B" (like the bursa in chickens) these cells were called B cells in contrast to thymus-derived cells which henceforth were called T cells.

In the beginning of this section it was stated that the knowledge of immunity and of the lymphoid system developed quite separately for a long period of time. In 1958 Medawar postulated the existence of what he called an "immunologically competent cell" which was defined as: "a cell which is fully qualified to undertake an immune response," though not having an inkling of what this cell might be, while several years before Florey had stated:

"The function of the lymphocyte is unknown. They can be observed after a day or two in areas of inflammation, but we have not the slightest notion why they are there. Congregated at the edge of the lesion, they have the appearance of phlegmatic spectators passively watching the turbulent activities of the phagocytes. Nothing of importance is known of the function of these cells other than that they move and that they reproduce themselves"[40,41] (see also Reference 13).

Only 10 years later, in 1968, the immunologically competent cell turned out to be a duality, one half being thymus-derived (T cells), the other being bone marrow (or in chickens: bursa-) derived (B cells), each responsible for a particular type of immune response leading to cellular or humoral immunity, though in some instances one would need the other to be fully capable of deploying its immune potential (T-cell-dependent antibody formation).

III. ORIGIN AND PRODUCTION OF IMMUNOLOGICALLY COMPETENT CELLS (ICC) WITH SPECIAL REFERENCE TO THE THYMUS AND T CELLS

In the previous section, ICC were identified as T and B cells, each with its own primary lymphoid organ as its source organ. As such, the bone marrow (BM) is the primary lymphoid organ for B cells and will be dealt with in Chapter 2. In this section special attention will be paid to the thymus as the primary lymphoid organ for T cells even though the thymus itself depends on a presumably continuous influx of cells from the bone marrow, prothymocytes, as precursors for T-cell differentiation processes within the thymus.

A. MICROANATOMY AND CELLULAR CONSTITUENTS OF THE THYMUS

1. Ontogeny and Microanatomy

Essentially, the thymus consists of two lobes originally derived from epithelial primordia of the third (and in some instances also the fourth) left and right pharyngeal pouches. Initially, these epithelial primordia contain a lumen (the thymopharyngeal duct) which, upon further differentiation, disappears to give rise to a solid mass of epithelial cells. During ontogeny the two thymic primordia migrate anterocaudally, eventually to join in the midline substernally in the mediastinum anterior overlying the heart and its large vascular trunk. At some time during this process the thymus becomes invaded by hematopoietic cells from which the first, i.e., cortical, thymocytes derive. In fact, at this stage of embryonic development the thymus is the only place where cells of a lymphoid nature can be detected. This observation led Beard (*vide supra*) to postulate that all lymphoid cells eventually derive from the thymus.[10]

Later, from the surrounding mesenchyme, septa begin to invade the mass of epithelial cells (now infiltrated with early thymocytes), penetrating to a certain depth and thereby defining future cortical and medullary areas. From the depth of the septa blood vessels infiltrate the thymus mass running parallel to the cortical surface. Eventually, capillary loops are formed in the cortex extending as far as the connective tissue capsule, from where they return to the corticomedullary junction. Separately, a vascular network develops in the now also developing medulla. In all instances these vascular structures remain separated from the thymic tissue proper by a basement membrane and a layer of epithelial lining cells also extending along the septa and the outer connective tissue capsule. By this time the thymus has obtained its lobular organization, which will persist during the remainder of its functional life.[42]

There are no indications that the thymus is provided with afferent lymphatics and the surrounding connective tissue capsule even seems to be devoid of any draining lymphatic vascular structures. Lymphatics have only been found at the corticomedullary junction and in the connective tissue containing perivascular spaces in the medulla. These lymph capillaries

eventually converge to form larger vessels and leave the thymic lobule along with the interlobular veins by way of the septa to reach the surface of the thymus gland from where, through afferent lymphatics, they drain into surrounding lymph nodes.

Innervation of the thymus is both of a parasympathetic (through branches of the right and left vagus nerve) as well as a sympathetic (from upper thoracic ganglia) nature. This may affect thymic structure and function both indirectly through effects on its vasculature, and directly through effects on epithelial cells and thymic lymphocytes, where appropriate receptors for relevant neurotransmitters have been demonstrated (for more detailed descriptions see References 43 and 44). In Chapter 5 interactions of the thymus with the CNS will be described.

2. Cellular Constituents

The vast majority of cells present in a normal young adult thymus are lymphoid in nature, most of these being thymocytes in various stages of differentiation. In addition, occasional B cells and mature T cells can be found especially in the perivascular spaces in the medulla, where under pathological conditions even follicular structures with germinal centers may develop.

The basic framework of the thymus, however, consists of a meshwork or reticulum of epithelial cells, with the thymocytes interspersed in the interstices between these cells and their cytoplasmic extensions. This epithelial reticulum can be roughly subdivided into two major types — cortical and medullary — differing in various aspects like MHC class II expression (cortical: always positive; medulla: variable in different species) and the delicacy of their cytoplasmic extensions (cortical: long, thin; medulla: coarser and more blunt). A more sophisticated classification, using electronmicroscopy, identified six different types, four cortical and two medullary.[45] Using monoclonal antibodies still another subdivision can be made by separating subcapsular/perivascular (type 1 cells) and medullary (type 5 cells) epithelial cells from the majority of the cortical epithelial cells (types 2, 3, and 4).[46]

The perivascular epithelial cells along with the basement membrane surrounding the cortical capillaries, which are of the continuous (nonfenestrated) type, together are thought to constitute the "blood-thymus barrier", preventing entry of antigens (self or foreign) into the thymus parenchyma.[47] However, in Section III.D a different view will be discussed. In addition to these essentially nonmobile nonlymphoid cells, i.e., the epithelial reticular framework, at least the presence of two types of nonlymphoid but mobile cell types should be discussed. By using the term mobile it is meant that these cells, like the lymphoid prothymocytes, enter the thymus from without, presumably via the bloodstream, either to exit again after a certain period of time or to die *in situ* and be replaced by newly incoming cells. The cell types referred to are (1) macrophages (in the rat recognized by the monoclonal antibody ED2) especially located in the cortex, and (2) interdigitating or dendritic cells located at the corticomedullary junction and throughout the medulla. The latter cell type, like the epithelial cells in the cortex, constitutively express MHC class II antigen.[48] The macrophages in the cortex are thought to play an essential role in eliminating the vast majority of not positively selected thymocytes dying through apoptosis.[49] It has been suggested that because of the advanced stage of decay (high endonuclease activity) of dying thymocytes before they are taken up by cortical macrophages, digestion of these phagocytized cells should not take long; thus explaining why, despite the profuse cell death occurring in the thymus cortex, which should make this area look like a "graveyard", histological signs of this process are virtually absent (J. James, personal communication).

Finally, mast cells are a regular feature closely associated with vascular structures, especially in the connective tissue capsule and septa, where they undoubtedly will play a role in regulating vascular flow at a microcirculatory level.

B. CELL KINETICS OF LYMPHOID (THYMOCYTES) AND NONLYMPHOID CELLS OF THE THYMUS PARENCHYMA

1. Nonmobile or Fixed Cells (Nonlymphoid)

Although the structure above the epithelial framework was considered to be nonmobile, recent evidence indicates that this may be less true than hitherto supposed. This does not mean that there is a stream of epithelial cells leaving the thymus to be replaced by new incoming cells, but as a population it seems to have its own dynamics, which partly are dictated by the cells embedded in its crevices.[46] In several instances, where the thymus is (partially) depleted of its population of lymphoid cells (as during treatment with cyclosporin A, adriamycin, or whole-body X-irradiation) or when this population never develops completely (as in SCID mice), the number of cortical or medullary cells may be affected. This does not seem to be the mere result of a more condensed packing of the cells now that spaces between the cells are no longer filled with lymphoid elements. Instead, this seems to result from lympho-stromal interactions which, to Van Ewijk et al.,[46] represent a symbiotic relationship in which T cells depend upon an intact thymic stroma for their maturation (as in ontogeny); in turn, the integrity of the stroma depends on the presence of (developing) T cells (as in the cases mentioned above; see also Reference 91). For example, the defect in medullary epithelial cells in SCID mice could be restored to normal by the i.v. infusion of normal bone marrow cells, giving rise to a fully competent population of maturing thymocytes, apparently providing an inductive signal for maturation of the medullary environment which in turn allowed terminal maturation to virgin T cells and their exit to the peripheral immune system.

2. Mobile or Nonfixed Cells (Lymphoid)

As early as 1963, Harris and Ford, using the T6-chromosome marker technique in neonatally thymectomized and subsequently thymus-grafted mice, noticed a stream of cells from the thymus graft to peripheral lymphoid tissues of the host.[50] When analyzing the chromosome make-up of the cells dividing in the thymus graft they observed a gradual shift from donor- to host-type mitoses. In a later series of experiments involving part body (lower half) X-irradiation and reconstitution with chromosome marked (T6T6) bone marrow cells, increasing numbers of T6T6 bone marrow-derived mitoses could be identified in the (nonirradiated) thymus, the first donor-derived cells being detected between 16 and 18 days post reconstitution.[51]

Using a single dose of adriamycin (ADR) injected i.v., gradual depletion of the thymus cortex could be observed, and this was complete by 7 days after ADR administration. ADR typically affects only blast-like cells leaving the majority of thymocytes intact; peripherally, mature recirculating B and T cells are not affected whereas bone marrow hematopoiesis is totally wiped out but for the bone marrow stem cells. In AO rats thus treated the first signs of thymus (cortex) regeneration were observed by day 16 and was virtually complete 7 days later. However, in BN rats regeneration was slightly retarded (first signs by day 19, full restoration by day 28) (Nieuwenhuis et al., unpublished).

Similar observations were later made by Kampinga et al. using a vascular thymus transplantation technique between two congenic rat strains carrying different allotypes of the RT7 marker (a leukocyte common antigen).[52] In these near-physiological experiments a vascularized thymus graft from an RT7.2$^+$ donor was grafted onto the carotid artery and jugular vein of an RT7.1$^+$ recipient. By using the appropriate monoclonal antibodies both the exit of donor-derived cells and entry of host-derived cells could be monitored. It appeared that as early as 11 days post-grafting, substantial numbers of donor-derived cells could be found scattered

all over the cortex. By 25 days all donor cells had been replaced by host-type cells. In these experiments no evidence was obtained for the existence (under these near physiological conditions!) of a long-lived intrathymic stem cell as postulated by others.

In the experiments from Harris and Ford et al., as well as in the ADR experiments, bone marrow hematopoiesis had to be restored to normal, at least to a certain extent, before prothymocytes become available to colonize the depleted thymus. Allowing a 7-day interval for this regeneration to take place, a lag time of 9 to 11 days still remains, as in the vascularized thymus transplant experiments, before actual entry into the thymus could be observed, which is a rather puzzling phenomenon. The process of T cell maturation from the earliest entry of detectable thymocyte precursors to the appearance of fully mature medullary type T cells apparently can be completed within approximately 7 days, which is compatible with the time needed for the thymus cortex to become depleted after blocking the input into the normal T cell differentiation process by a single dose of ADR.

3. *Mobile or Nonfixed Cells: Nonlymphoid*

Both from BM reconstituted radiation chimera experiments and from the vascularized thymus transplant experiments, evidence was obtained that both cortical macrophages as well as medullary interdigitating or dendritic cells constitute mobile elements in that they are replaced by incoming cells apparently derived from the bone marrow.[48,52] For IDC a turnover of approximately 28 days was observed, which was in the order of magnitude of thymocyte replacement. In the radiation chimera experiments this turnover time also applied to dendritic cells elsewhere, such as in spleen and lymph nodes as well as in nonlymphoid organs.[53] Efflux of donor thymus-derived interdigitating cells (IDC) to the periphery, however, could not be observed, indicating (1) that replacement in the periphery is independent of the thymus, and (2) upon entry into a particular lymphoid organ dendritic cells (DC and IDC) presumably have reached their final destination. The functional significance of the relatively short life span of DC remains to be established.

For macrophages a much longer turnover time was observed in the vascular thymus transplant model: by 60 days after grafting only 60% had been replaced by host-derived incoming cells; moreover even by 120 days a considerable number of donor-type macrophages were still present.[52] In lymph nodes, effete macrophages, e.g., in a germinal center, are phagocytized by lymph-borne incoming cells resulting in a continuous turnover of germinal centers macrophages. Apparently the rate of turnover in the thymus is much slower, perhaps because lymph- or blood-borne macrophages are not easily attracted to the thymus parenchyma.

C. INTRATHYMIC T CELL DIFFERENTIATION AND MHC RESTRICTION

Recently some excellent reviews have appeared describing the process of intrathymic T-cell differentiation in full detail.[42,54,55] Here attention will be focused on several elements only, so as to provide a sufficient background to understand the intricacies of the process leading to the production of a useful set of T cells capable of participating in peripheral immune responses while potentially harmful (autoreactive) T cells are eliminated.

From the experiments by Miller and Mitchell (1968) it appeared that the role of the thymus was to provide the organism with cells capable of recognizing antigen ("antigen reactive cells") to subsequently help bone marrow-derived antibody-forming cell precursors ("antigen sensitive cells") to actually form antibodies.[38] For this recognition process T cells would need an antigen receptor, which by some was considered to be akin to a classical immunoglobulin molecule, like the antigen receptor on B cells and called IgT.[56] Later it

turned out that the T-cell receptor for antigen (TCR), although belonging to the Ig supergene family, was a molecule in its own right consisting of a disulfide-bonded heterodimeric glycoprotein composed of two chains termed α and β (while some T cells express γ and δ chains), the two chains together forming an antigen binding site through which antigen, if presented in the correct way, could be recognized.[42]

B cells are capable of recognizing antigen in a soluble form and are mainly concerned with extracellular antigens. T cells, however, can only recognize antigen — in present-day terminology — in the context of an MHC class I or class II molecule — where the antigen or a relevant part of it in the form of a small oligopeptide is presented in the groove formed by the two MHC molecule constituting chains.

As such, naive T cells can only recognize antigen that has been "processed" through an intracellular pathway before it — along with an MHC molecule — is presented at the surface of the "antigen presenting cell". For cytotoxic T cells these can be virally infected class I positive cells (all nucleated cells in the body are class I positive), whereas for helper T cells antigen has to be presented by MHC class II positive dendritic cells (DC).[1]

For B cells to recognize, the antigens of an invading microorganism, it is not essential in which individual of a certain species they recirculate. A T cell has to take into account that the antigen will be presented in the context of *that* individual's MHC molecules which, due to the polymorphism of the MHC system, will be quite different from those in other individuals. This fact has at least the three following implications: (1) within the genome of a T cell-to-be (a prothymocyte) information should be stored for such a diversity of TCRs that in principle all MHC molecules occurring in any one individual within that *species* can be recognized; (2) expression of all this information in one particular individual without further selection would lead to a massive redundancy of useless T cells, swamping the few T cells with fitting TCRs; and (3) selection within one particular individual of only those T cells with a fitting TCR capable of recognizing "self" MHC molecules might lead to over-representation of potentially autoreactive cells. Thus measures should be taken either for a further selection process or, in case autoreactive T cells would be allowed to exit from the thymus, activation in the periphery should not lead to the successful production of autoreactive effector cells; some form of suppressor mechanism aimed at keeping activated autoreactive T cells in check should be present. With these implications in mind the different phases of intrathymic T-cell differentiation may make more sense than they would seem to make at first sight.

Bone marrow-derived prothymocytes are thought to enter the thymus through blood vessels at the corticomedullary junction or in the subcapsular area of the cortex.[57] Factors determining this (site of) entry still remain elusive, although β_2 microglobulin as a chemoattractant has been implied.[57] At this stage the Thy-1 marker (formerly θ antigen) is upregulated and cells are $PgP1^+$ (CD44), $CD4^-$, $CD8^-$, $CD3^-$, and TCR^-. The bulk of cell proliferation would seem to occur at this stage, although during later stages cell proliferation is not totally absent. By the end of this amplification process, during which the cells gradually shift from the subcapsular area to deeper cortical areas, these early precursors begin to express TCR, first intracytoplasmatically and later, although at low density, along with the surface CD4 and CD8 accessory molecules.

The vast majority of cells in the cortex are of this $CD4^+CD8^+TCR^{lo}CD3^+$ phenotype and are enmeshed in the interstices of type 2 and 3 (cortical) epithelial cells, which according to some authors, constitute typical microenvironments termed thymic nurse cells.[58] Presumably at this stage the first selection process occurs, i.e., cells are positively selected to survive if binding of the TCR along with CD4 and/or CD8 accessory molecules to the class I and II positive surrounding epithelial cells is sufficiently effective in transferring signals to prevent apoptosis (programmed cell death). Thus the vast majority of cells with TCR that do not fit the MHC molecules of that particular individual are not allowed to live and are quickly eliminated

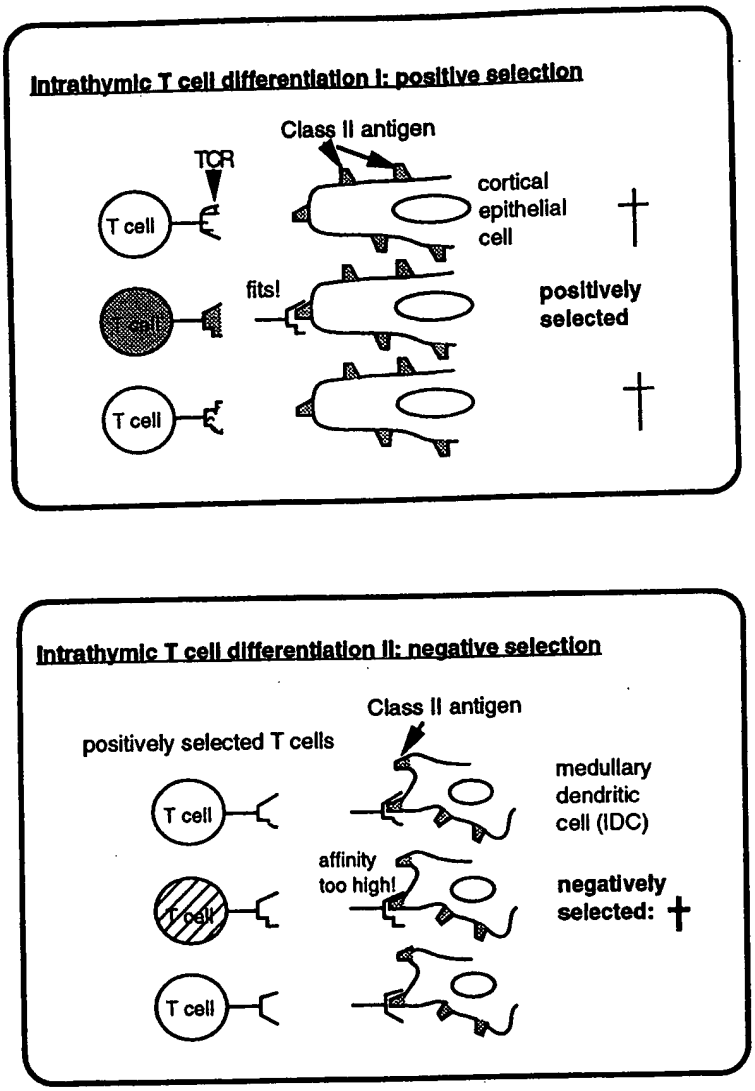

Figure 1 Positive and negative selection of thymocytes.

by the omnipresent cortical macrophages (Figure 1). Presumably the fitting of a TCR to either "host" MHC class I or class II molecules determines the downregulation of the noncorresponding accessory molecule, i.e., a TCR fitting to a class II molecule results in downregulation of CD8, and the same would hold for class I and CD4. Eventually, those cells from the population of $CD4^+CD8^+TCR^+CD3^+$ cells that have been positively selected then divide into single positive $CD4^+$ or $CD8^+$ cells with further upregulation of the TCR CD3 complex. By this time these cells will have passed through the corticomedullary junction into the medulla. Before exiting, however, a final check is made on the appropriateness of the T cells to be exported, i.e., their affinity for self-MHC molecules (the basis for positive selection) is tested in contact with medullary dendritic cells which are both class II and class I positive. When affinity is too high (which would entail the risk of peripheral activation without the extra stimulus of a foreign peptide in the groove of the presenting MHC molecule and thus result in autoreactive effector cells) the cells are signaled to die (elimination of "forbidden" clones or clonal deletion). This process is called negative selection.

The picture described above has emerged from numerous experiments involving radiation chimeras as well as transgenic animals for particular TCR Vβ and/or Vα chains. For illustration, one particular type of experiment will be described from which restriction of T cells to "host" MHC molecules was concluded. T cells from strain A mice are quite capable of cooperating with B cells from strain A mice in staging a T-cell-dependent antibody response. Similarly, *individual* T cells from (A × B) F_1 mice were found to cooperate with B cells from either A or B strain mice, but not both. However, when bone marrow cells from (A × B)F_1 mice were "educated" in an X-irradiated A strain mouse only cooperation with A strain B cells would follow, but not with B strain B cells ("restriction" to A strain MHC class II only). Conversely, A strain bone marrow-derived T cells in an (A × B)F_1 strain mice were found to cooperate with either A or B strain B cells, as if the bone marrow had been derived from an (A × B)F_1 hybrid.[59,60] This might be interpreted as an "expansion" of the repertoire of A strain bone marrow thymocyte precursors! The latter type of experiment clearly indicates that, within the genome of bone marrow cells from *any* mouse strain, a potential repertoire of TCR is stored which is capable of interacting with *any* MHC molecule occurring within the *species* mouse. Also from these radiation chimera experiments it emerged that the restricting element within the F_1 "educating" thymus are the cortical epithelial cells and not medullary dendritic cells, as these, within a rather short period of time (see above), will be replaced by donor bone marrow-derived DC expressing only donor (A strain) type MHC class II. It still remains a mystery why in these A → (A × B)F_1 chimeras, where negative selection for B type MHC molecules cannot occur due to the absence of B type DC in the medulla, graft vs. host disease resulting from high affinity B type fitting T cells does not occur. One solution might be that in the periphery B type DC are absent, which might constitute a primary target in triggering graft vs. host disease.

D. INTRATHYMIC T CELL DIFFERENTIATION AND (SELF) TOLERANCE INDUCTION

In the previous section it was described how the processes of positive and negative selection would result in the production of a population of $CD4^+$ or $CD8^+$ T cells with self-MHC restricted TCR and devoid of self-MHC (+ self peptide) autoreactive clones. The question remains whether this population also lacks T cells with TCR that in a self MHC restricted way might recognize self non-MHC molecules. Apparently this may not necessarily be the case. A major question then would be if these cells exist, would they be harmful. It has been argued that elimination of T cells with high self-MHC TCR affinity in itself would be sufficient for complete self tolerance, the argument being that self-MHC + self peptide (on APC) would constitute a signal too weak for the remaining T cells that would need self-MHC + foreign peptide to become activated. Although MHC molecules either on epithelial cells or dendritic cells presumably have their grooves filled with some self peptide, it is difficult to envisage that in this way *all* potential self peptides which are not constitutively expressed within the thymus could be presented.[61]

Formal proof now exists that for self non-MHC molecules, provided they are presented within the appropriate context, intrathymic clonal deletion also would be operational. This evidence was obtained from experiments involving animals transgenic for a specific set of α and β chains of a TCR recognizing H-Y antigen, which in male (but not in female) mice is constitutively expressed along with class I molecules. It was found that T cells carrying the transgene TCR were predominant in female but absent in male mice, where they apparently were clonally deleted.[62] Even so, for self antigens not constitutively expressed within the thymus, clonal deletion would be difficult to envisage since the thymus is considered to be impenetrable to (self) antigens because of the existence of the so-called "blood-thymus barrier" (BTB), as mentioned earlier.[47]

However, data from our own experiments indicate that the thymus parenchyma, especially the cortex, despite the presence of a BTB, may be bathed in (low molecular weight) self proteins. In a series of experiments where young adult rats were injected with monoclonal antibodies reacting with particular cell surface markers in the thymus (like class II and a pan-T cell marker), it was found that shortly after i.v. injection these antibodies penetrated the cortex from the capsule, and subsequently, along with a flux of tissue fluid, moved centripetally towards the corticomedullary junction. Because these antibodies reacted with cell surface markers present in the cortex they could build up to a sufficient concentration to be detected, in contrast to other tracer proteins that had been used before which never could be detected inside the thymus, from which observation the idea of a BTB originated. This route of antigen access, bypassing the BTB and originating in the thymus capsule and its fenestrated capillaries (see Section III.A.1), has been termed "the transcapsular route" (for further discussion on this topic see References 63 and 64). Disturbance of the BTB and this transcapsular route by the injection of estradiol in rats was demonstrated by Zapata et al. and might have implications for a higher propensity of females to certain types of autoimmune disease[65] (see also Reference 66).

Finally, some attention should be paid to the induction of allotolerance, especially as this might be of clinical relevance. Experimentally, allotolerance can be easily induced by constituting (semi)-allogenic radiation chimeras. A rather straightforward procedure is to lethally X-irradiate A strain mice or rats and to reconstitute these animals with $(A \times B)F_1$ bone marrow cells. Upon recovery of immune responsiveness after several weeks, animals thus treated will accept skin grafts from a B strain donor which otherwise would have been rejected, but not from a third party. When this model was further analyzed it could be shown that the new population of dendritic cells in the thymic medulla expressing both A strain and B strain MHC class II molecules essentially should be considered the tolerogenic element responsible for clonal deletion of B strain alloreactive T cells. Evidently the recipient's thymus epithelial cells could not have been instrumental in bringing about this kind of allotolerance as they only express host type A strain MHC class II molecules.[67]

Whereas antigen in peripheral lymphoid organs induces various kinds of immune responses, apparently the intrathymic presentation of either self- or even foreign antigens might lead to the induction of a state of tolerance, either by clonally deleting potentially reactive cells or by some other mechanism like induction of anergy or even suppressor cells.

By the late 1960s foreign antigens, either in soluble form or bound to the cell surface, were deliberately injected intrathymically by several authors to test the possibility of intrathymic tolerance induction.[68,69] More recently, Posselt et al., from the group of Naji, intrathymically transplanted allogeneic pancreatic islets into diabetic recipients along with a short course of immunosuppressive treatment. This procedure not only resulted in prolonged survival of the transplanted islets but also in donor-specific tolerance to subsequent extrathymically (i.e., under the kidney capsule) transplanted islet allografts.[70]

Odrico et al. and later Goss et al. showed that comparable results could be obtained by the intrathymic injection of unfractionated donor bone marrow or spleen cells using cardiac allografts as a readout system for donor-specific allotolerance.[71,72] Results from our own group indicate that this donor-specific allotolerance by transfer of spleen cells from the thus tolerized animal can be conferred upon a syngeneic recipient, resulting in prolonged cardiac allograft survival (>200 days) and indicating that not clonal deletion but the induction of suppressor cells might be responsible for this state of tolerance.[73] The exact mechanisms of the intrathymic processes resulting in tolerance induction, however, remain to be elucidated.

A major drawback of the above-described procedure, however, could be that, clinically, by the time the thymus would be needed for tolerance induction to allow some sort of allotranplantation (heart, kidney, islets) this organ may have atrophied to a state where

tolerance induction is no longer feasible. However, experiments by grafting old-age thymus back into young recipients have shown that the thymus is still intrinsically capable of functioning normally as these thymuses regained their normal appearance (J. Kampinga, personal communication). Presumably, the hormonal condition in the younger animal favored thymus regeneration. Moreover, experiments using leutinizing hormone releasing hormone (LHRH) injected intrathymically either systemically or directly resulted in regeneration of old-age thymuses, showing normal T-cell differentiation and even production of a new set of T cells as witnessed by the appearance in the periphery of increased numbers of recent thymic migrants. Thus, by hormonally manipulating the thymus, it might be possible to bring the thymus back to a stage where it can be used for successful intrathymic tolerance induction[74] (and J. Kampinga, unpublished data).

IV. T CELLS AND PERIPHERAL IMMUNE RESPONSES

In the first section of this chapter the immune system was subdivided into an innate (nonspecific, nonadaptive) immune system and a cognate (specific and adaptive) immune system. It was also mentioned that the innate immune system, in principle, could spring into action in virtually every niche of the body due to local vascular changes resulting from the action of locally produced or released toxins and cytokines, thereby directing cells of the innate immune system to the site of action. In this respect any polymorphonuclear granulocyte will do, irrespective of the type of invading bacteria (Figure 2).

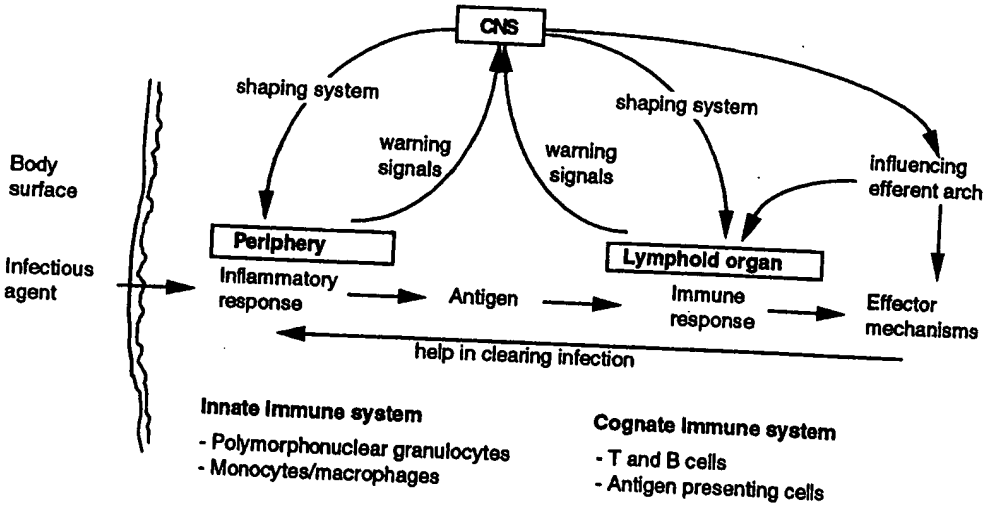

Figure 2 An overview of the immune system.

For the cells of the cognate immune system, with their highly specific repertoire, it is essential to increase the chances that a particular cell of this system meets with "its" antigen that this meeting takes place in a highly organized manner. To this end, secondary lymphoid organs have developed with a dual purpose:

1. The bringing together of antigen and naive lymphocytes, and
2. To support the clonal expansion of activated cells and their differentiation into effector/memory cells.

For the first purpose secondary lymphoid organs need special provisions (1) to collect antigen from virtually every site in the body and to direct this to their specialized microenvironment, and (2) to recruit lymphocytes from the circulation in a continuous manner to temporarily form part of the population of immunologically competent cells assembled in that organ before passing on to another station to perform the same task elsewhere (recirculation and redistribution). As such, secondary lymphoid organs are best viewed as highly structured "meeting places" between antigen and cells of the cognate immune system, i.e., T and B cells.

A. MICROANATOMY AND CELLULAR CONSTITUENTS OF PERIPHERAL LYMPHOID ORGANS

In this section attention will be focused on lymph nodes as typical examples of secondary lymphoid tissue. Most of this description also holds for the spleen and mucosa-associated lymphoid tissues like Peyer's patches, etc. with the exception of the way antigen is brought to these tissues. In the case of the spleen, blood-borne antigens will reach its lymphoid tissue proper through vascular channels, whereas for Peyer's patches, etc. antigens first will have to penetrate the mucosal lining (special M cells may be instrumental in this process) before reaching the assembled lymphocyte population in their specially assigned areas.[75] For lymphocyte recruitment, provisions in Peyer's patches are essentially the same as in lymph nodes, whereas in the spleen with its open blood circulation the "homing" of lymphocytes (T and B cells) to their respective areas is regulated in a somewhat different way, which is beyond the scope of this chapter.[76]

1. Microanatomy

During ontogeny lymph nodes develop at genetically predetermined sites as local expansions of the developing system of lymph channels. A meshwork of mesenchymal reticulum cells form the basic scaffolding which, once the nodes become vascularized, is infiltrated with lymphoid and nonlymphoid elements. In a well-developed lymph node, a dense cortical area and a more loosely structured medulla consisting of strands of reticular tissue and lymph-containing sinuses, can easily be discriminated.

The *cortical* area itself can be subdivided into an outer cortex, containing follicular structures (B cell areas) — either primary, predominantly consisting of densely packed B cells, or secondary, consisting of (the remnants of) a germinal center surrounded by a mantle zone (lymphocyte corona) of B cells — and into a subjacent paracortical area (or deep cortex) predominantly consisting of densely packed T cells.

Each lymph node is surrounded by a connective tissue capsule which at various sites is penetrated by afferent lymphatics draining into the subcapsular sinus, from where lymph flows through the meshwork of reticulum cells and the lymphocytes contained within it, eventually to reach the medullary sinus and to leave the node, in its hilar area, by its efferent lymphatic(s). The afferent lymphatics originate in the periphery as open-ended lymph capillaries to collect tissue fluid and possibly cells and materials contained within it, to be transported from this drainage area to the lymph node upstream.

In the hilar area, one or two arterioles and veins enter and leave the node. Within the nodes the arterioles branch to give rise to a capillary network in the outer cortex (vascularization of the follicles) from where the blood drains into "postcapillary venules" or "high endothelial venules" (HEV) because of their almost cuboidal endothelial lining. These postcapillary venules merge to form larger venules that exit from the node along with its efferent lymphatic.

2. Cellular Constituents of the Lymph Node Parenchyma

Apart from the basic framework of reticulum cells, which only by special staining procedures can be detected, the bulk of the cells of a lymph node consist of mature but still naive, i.e., antigen inexperienced, T and B cells. Each of these cell populations is localized in a particular area as already mentioned above. The processes that underlie the homing to and temporary stay within these areas still remain obscure, but cell-cell and cell-matrix interactions through adhesion molecules of the integrin family appear to play a major role.

This subdivision into B- and T-cell areas is not absolute since in B-cell areas a special subset of T cells can be found, especially in secondary follicles containing germinal centers, where they are thought to play a role in the regulation of B-cell differentiation processes (like affinity maturation and Ig isotype class switching).[77,78] Also in T-cell areas, especially around the HEVs, B cells can be found that have just extravasated through the walls of these structures. As such, B cells have to migrate for at least part of their route to the outer cortex through T-cell territory. In an antigenically stimulated lymph node both T and B immunoblasts can be found scattered among the other resting cells. Especially in the medullary strands, more mature forms of B immunoblasts like plasma cells can be found.

In addition to these *lymphoid* elements several types of *nonlymphoid* cells contribute to the specialized microenvironment of these secondary lymphoid tissues. In follicular structures follicular dendritic cells (FDC) can be found, both in primary as well as in secondary follicles. These cells have Fc and C3 receptors on their surface through which they can bind antigen-antibody complexes and "present" these for a prolonged period of time to surrounding B cells, especially once a germinal center has formed within a primary nodule.[79] In the paracortical area, intermingled with surrounding T cells, the interdigitating (dendritic) cells or IDC, which were described for the first time by Veldman in 1970, are a striking feature.[30] Both FDC as well as IDC can be detected using special monoclonal antibodies, indicating that these cell types are two completely different entities, which is witnessed by the fact that IDC, in contrast to FDC, constitutively express high amounts of MHC class II antigens.[80]

Lining the sinuses (subcapsular, paratrabecular, and medullary) highly phagocytic macrophages constitute a normal component representing the "filter" function of lymph nodes with regard to the lymph flowing through the node. These cells can eliminate cellular debris, dead bacteria, etc. originating from the peripheral drainage area. A particular type of macrophage is usually found in active germinal centers where they play a role in the elimination of some of the B cells generated in a germinal center. During this process activated B cells (centroblasts) proliferate and also undergo somatic hypermutation, resulting in changes in the sIg expressed by these cells once they mature into centrocytes.[81] Unless these cells can contact the FDC and the Ag-Ab complex presented on its surface by a proper fit of their sIg, these cells are destined to die through apoptosis.[82] Dying cells are taken up by germinal center macrophages where whole cells or nuclear remnants can be detected as "tingible bodies" inside their cytoplasm. Hence, these cells have been called: "tingible body macrophages".

B. KINETICS OF THE VARIOUS CELLULAR CONSTITUENTS CONTAINED WITHIN A LYMPH NODE

1. Nonmobile or Fixed Cells (Nonlymphoid)

Clearly, the basic framework of reticulum cells is essentially nonmobile in nature. However, they must be adaptable so as to accommodate the various changes in an antigenically stimulated lymph node. Although for some time this framework of reticulum cells was considered as a rather inert system, new data indicate that by influencing the composition of

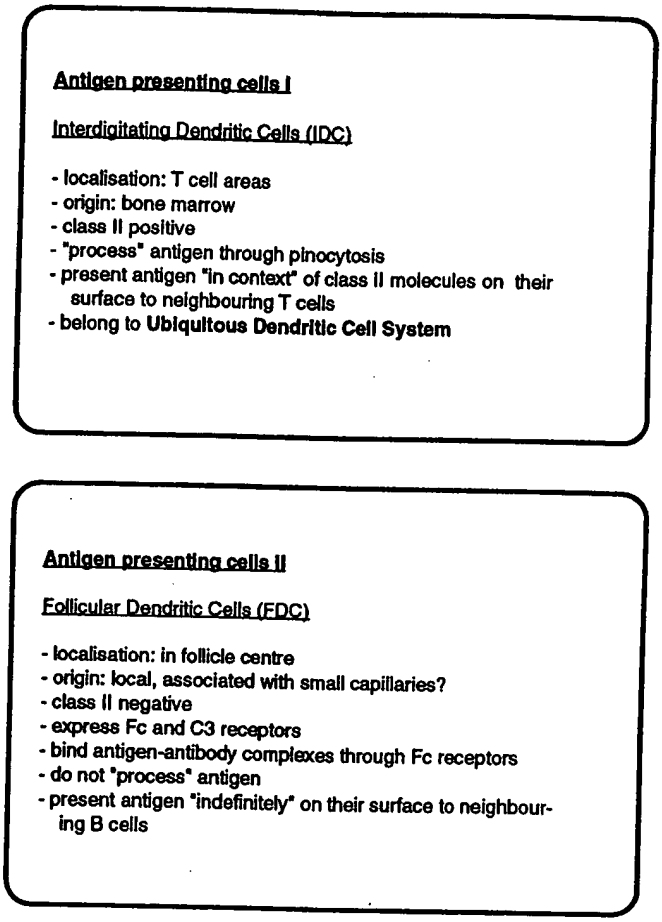

Figure 3 Antigen-presenting cells.

the extracellular matrix (of which they are the sole producers) migratory processes of other cells within the node might be regulated. As such, these cells may be under local or systemic hormonal control.

The follicular dendritic cell (FDC) also belongs to the nonmobile group of cells (Figure 3). Presumably FDC originate from local precursors which have not been identified.[80] The possibility exists that they may derive from mesenchymal elements surrounding the capillaries in the outer cortical network, at the time of the development of (primary) follicles. Speculating, one might say that due to factors carried by the lymph from the periphery, some mesenchymal elements change into FDC, further stimulating the local accumulation of B cells thus giving rise to a primary follicle. Essentially, follicular structures can develop anywhere, especially at sites of chronic inflammation. These follicles then are found both qualitatively as well as quantitatively to contain normal (numbers of) FDC. However, antigenic stimulation cannot be the driving force behind follicular development since in germfree animals (primary) follicles also develop normally.

2. *Mobile Cells (Lymphoid)*

As mentioned in the previous section, the capillary network of the follicles at the transition into the paracortical area changes into high-endothelial venules (HEV). In 1925 Schulze (see Section II.B) observed lymphoid cells in the walls of these structures, and in the experiments by Gowans and ourselves these HEV were found to be the site of entry of both T and B cells

into the lymph node parenchyma.[8,20,83] From here, B cells will move on, eventually to reach the follicles in the outer cortex (primary follicles and mantle zone of secondary follicles). After a short stay of approximately 24 to 48 h they will move on towards efferent lymph (the precise route as yet unknown) as part of the continuous recirculation and redistribution process. Likewise, T cells will stay in the paracortical area for some 12 to 24 h and eventually leave through the efferent lymphatic. These processes of extravasation and migration through the node's parenchyma is highly regulated both by ligands on the surface of T and B cells ("selectins") and special adhesion molecules on the endothelial surface ("addressins") as well as by cell-cell and cell-matrix interactions within the node itself.[84] After i.v. administration of a single dose of corticosteroid hormone (Solumedrol®) the number of cells coming out of the thoracic duct was reduced to virtually nil. After some 6 h, when the effect of the hormone waned, cellular output returned to normal. As entry into the node was not blocked by this hormone treatment, apparently the cells were somehow trapped within the node's parenchyma. Whether this was due to changes in surface make-up of the lymphoid cells or to changes in the framework reticulum could not be assessed at that time (Nieuwenhuis et al., unpublished data).

By surgically removing the afferent lymphatics, Hendriks et al. could show that this resulted in the loss of the high endothelial character of the postcapillary venules, which returned to normal when lymphatic drainage into the node had regenerated several weeks later. Thus entry into the node and perhaps even selection of specific subsets into the node can be influenced by factors (humoral or cellular) produced in the periphery.[85] This might, at least to a certain extent, explain why effector cells (cytotoxic T cells or plasma cells generated in a lymph node draining a particular skin area or in a Peyer's patch) show a preference to home to the skin or to the mucosal lining of the gut, respectively (for further discussion of this topic see Reference 86).

3. Mobile Cells (Nonlymphoid)

The two remaining cell populations, i.e., the interdigitating cells (IDC) and the macrophages, in a way may be considered as mobile elements, even though the lymph node for these cell types may constitute the end of the road.

Interdigitating cells, as found in the paracortical area, have a limited life span (approximately 4 weeks), eventually to be replaced by new cells coming in through the afferent lymphatics, where IDC precursors have been identified as so-called "veiled cells".[87] These cells either derive from the Langerhans cells in the epidermis or from the dermal dendritic cells. Eventually, these cells all are bone marrow-derived and may be said to be part of the ubiquitous dendritic cell (UDC) system (Figure 4). As mentioned before, dendritic cells can also occur in the blood and within the parenchyma of various organs and tissues (kidney, pancreas, heart, skin, gut mucosa, etc.). That means that these cells can be found everywhere in the body (ubiquitous = occurring everywhere) and presumably constitute a functional system which possibly belongs to the mononuclear phagocyte system (MPS). The MPS thus would contain two subsets of cells: (1) real macrophages, specially geared to phagocytose, and (2) the population of UDCs, capable of phagocytosing only to a limited extent although sufficient to process and present the degraded phagocytized material in the context of MHC class II which is constitutively expressed by these UDCs.

As to lymph node *macrophages*, not much is known about the kinetics of these cells apart from the fact that presumably the vast majority enter the node through afferent lymphatics. Efferent lymph contains very few macrophages indicating that most of the cells, after an unknown amount of time, die within the node. Indeed, evidence exists, e.g., that newly incoming macrophages may phagocytose obsolete TBMs thus forming a new population of TBMs. Macrophages do not constitutively express MHC class II molecules, although when appropriately stimulated they may do so.

The Ubiquitous Dendritic Cell System I

UDC versus FDC

- two types of dendritic cells:
  - = FDC are restricted to follicular structures only and are of local origin
  - = UDC can be found "everywhere" i.e. in blood, connective tissue, epithelia, parenchymatous organs, afferent lymph and T cell areas and are originally bone marrow derived
    ("ubiquitous" = occurring everywhere)
    Presumably they are part of the Mononuclear Phagocyte System (MPS)

The Ubiquitous Dendritic Cell System II

Phenotypical appearances of UDC

- blood - - - - - - - - - - - - - dendritic cells
- connective tissue - - - - - - - dendritic cells
- epithelia (e.g. skin) - - - - Langerhans' cells
- thymic medulla - - - - - - - - dendritic cells
- parenchymatous organs - - - "passenger leukocytes"
- afferent lymph - - - - - - - - "veiled cells"
- T cell areas - - - - - - - - - interdigitating cells

Functional capacities

- uptake, processing, transport and presentation of antigens

The Ubiquitous Dendritic Cell System III

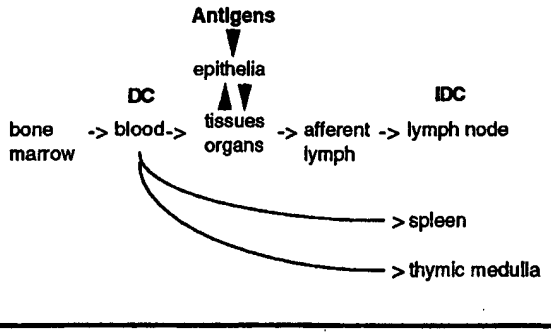

Figure 4 The ubiquitous dendritic cell system.

C. T CELL DEPENDENT AND T CELL INDEPENDENT IMMUNE RESPONSES

As mentioned in the introduction to this section, peripheral or secondary lymphoid tissues may be seen as highly specialized microenvironments facilitating the meeting between antigen and immunologically competent cells, be they T cells, B cells, or both, resulting in the various

types of immune responses. Essentially, two types of immune responses can be envisaged as leading to cellular or humoral immunity as well as immunological memory through the production of effector/memory cells and/or molecules. In these immune responses, T cells are either involved directly (cellular immunity) or indirectly through helping B cells. For some antigens such as lipopolysaccharides, B cells can be activated directly without needing T-cell help, resulting in T-cell-independent antibody formation, usually only of the IgM isotype, without further class switching. Also, as these antigens do not induce germinal center formation (which is a T-cell-dependent phenomenon) no memory B cells are formed. (For further discussion see Chapter 2.)

1. Cellular Immunity

Skin painting with a chemical sensitizer like DNCB or oxazolone or the application of a foreign skin graft in the drainage area of a lymph node, results within several days in the activation of virgin T cells with the appropriate TCR in the paracortical area of the draining lymph node.[29,30] This T-cell activation is witnessed by the transformation into T immunoblasts, which can be seen scattered throughout the paracortical area. A second effect of this activation is that these transformed T immunoblasts start to proliferate (detected by the incorporation of ^{3}H-thymidine). Resulting from locally produced cytokines by these activated T cells, vascular changes occur thereby increasing the recruitment of more (T) lymphocytes into the node's parenchyma, which is clinically observed as a swelling of the node. After several days this wave of cell proliferation wanes, with T immunoblasts further differentiating into smaller T-effector/memory cells.[88] This differentiation process involves changes in the capacity of these cells to secrete a different array of cytokines as compared to virgin T cells as well as a change in homing receptors (adhesion molecules) thus facilitating the extravasation in nonlymphoid tissues. These cells start to leave the lymph node through the medullary sinuses to exit through the efferent lymphatic, eventually to reach the systemic circulation. At the site of the graft (which the host's vascular system has now invaded), these cells will recognize the alloantigen(s) that initially induced the response. Recognition may lead to local activation of the clotting cascade, which evenutally will result in necrosis of the graft and thus its rejection.

The effect of painting with a chemical sensitizer will not be detected until after a second challenge, where locally induced inflammatory reaction, vascular changes will result in the recruitment of effector/memory cells generated in the initial (or "priming") immune response, thus showing the change in homing potential of these cells but not in their specificity.[86] Activation of these effector/memory cells induces the secretion of a different set of cytokines (including TNF-α) attracting other inflammatory cells from the circulation and leading to macrophage activation. As this response takes some time (24 to 48 h) to develop, being dependent upon cell influx and activation in contrast to antibody (IgE) mediated atopic reactions, it has been called delayed type hypersensitivity.

2. Humoral Immunity

For T-cell-dependent antibody production, the initial processes are the same as described above, i.e., that antigen-laden dendritic cells arrive through the afferent lymphatics in the paracortical area where they transform into interdigitating cells (IDC). These professional antigen presenting cells are the only cells capable of activating naive CD4$^+$ (Th0) T cells. The local production of cytokines (IL-1) by IDC and presumably cytokines coming into the node from the afferent lymphatics produced at the site of antigen deposition, cause naive (Th0) cells to differentiate into Th2 cells capable of "helping" B cells recognize different epitopes on the antigen (Figure 5).[87]

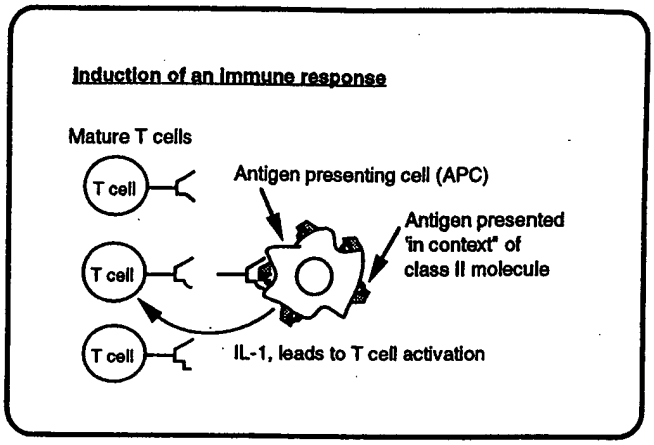

Figure 5 Induction of an immune response.

Types of specific immune responses I

T cell activation

- activation of Th-1 cells (CD4+) leads to activation of cytotoxic CD8+ cells: **cell mediated immunity**

- activation of Th-2 cells (CD4+) leads to activation of B cells and antibody formation: **humoral immunity**

In both instances T cells transform into T cell immunoblasts, however, with different cytokine profiles: e.g. IL-2 and IFN-g (Th-1) versus IL-4, IL-5, IL-6 and IL-10 (Th-2)

Site of reaction: T cell areas

Types of specific immune responses II

B cell activation

- T cell *independent:* induced by e.g. bacterial capsular antigens (lipopolysaccharides)

- T cell *dependent:* APC-antigen stimulated B cells aided by a.o. IL-4 from activated Th-2 cells transform into plasmablasts, that divide and develop into a clone of antibody producing and secreting plasmacells: **plasmacellular reaction.**
  Some activated B cells do not follow this differentiation pathway but -inside a follicle centre- transform into centroblasts giving rise to a Germinal Centre leading to B memory cell formation: **germinal centre reaction**

Figure 6 Types of specific immune responses.

The observation that B cells enter the node through HEV in the paracortical area and have to traverse T cell territory, at least for some distance, may be instrumental in selecting (and activating) potentially reactive B cells. If so, these B cells interrupt their normal migratory route to transform into plasmablasts, a process that is highly stimulated and regulated by the

cytokines (IL-4, IL-5, IL-6, IL-10) produced from Th0 → Th2 transformed T cells, with IL-4 stimulating a switch to IgG and IgE synthesis and TGFβ to IgA.

The plasmablasts, after several cycles of cell division, will transform into mature plasma cells, leaving the node through the efferent lymphatic heading for the circulation. Spleen and lymph node plasma cells eventually migrate to the bone marrow, still producing antibodies that may extravasate at the site of the intruding microorganism to assist in the clearing process by enhancing phagocytosis through locally activated macrophages, expressing Fc (and C3) receptors.[89] For Peyer's patches (and presumably also mesenteric lymph nodes), these cells eventually will home to the gut mucosa to locally secrete IgA antibodies, which may be excreted into the gut lumen through gut epithelial cells.

In addition to B effector cells, B memory cells are produced in germinal centers that develop synchronously with the PCR but tend to persist for several weeks. This results from prolonged antigen exposure on the surface of the FDC where antigen-antibody complexes localize several days after the PCR and GCR are well on their way (Figure 6). (For further discussion of B cells and GC see Chapter 2.)

3. Cellular Vs. Humoral Immunity: A Role for Antigen Presenting Cells?

A major question still remains as to the factors involved in determining whether cellular or humoral immunity will develop in response to a certain antigen. In the case of a foreign skin graft both cellular and humoral immunity develop, each playing its own role in the eventual rejection of the graft. In these responses apparently both $CD4^+$ Th1, and Th2 cells are formed. In the case of DTH, predominantly $CD4^+$ Th1 cells are produced which themselves can elicit a DTH response upon secondary challenge, although $CD8^+$ cells activated through these $CD4^+$ Th1 cells may also be instrumental. In other instances the development of $CD4^+$ Th2 cells predominates where the cytokines produced by these cells not only assist in B cell differentiation but also (e.g., IL10, possibly through inhibiting macrophage derived IL-12 secretion) downregulate the development of Th1 cells.[90]

With the DC as the only professional APC capable of stimulating naive (Th0) $CD4^+$ cells, factors like cytokines produced by macrophages and other cells, either locally or "upstream" in the drainage area of the node, apparently determine the balance between Th1 and Th2 cells with each of these mutually downregulating the development of the other. Thus the history of antigenic exposure as well as the way these antigens are handled by antigen presenting cells might play a role as to whether immune responsiveness may be biased either towards Th2 responses (humoral immunity) or Th1 responses (cellular immunity). This might explain why some strains of rats are sensitive to the induction of cell-mediated autoimmune disease, i.e., Lewis rats for experimental allergic encephalomyelitis (EAE) or BN rats for $HgCl_2$ induced immune complex glomerulonephritis (ICGN). One type of response virtually excludes the other type of response (Lewis rats cannot be induced for $HgCl_2$-ICGN, nor can BN rats be induced for EAE). A puzzling question still remains as to why (Lew × BN)F_1 hybrid rats are sensitive to the induction of either type of autoimmune disease, whereas AO rats are sensitive to neither (Nieuwenhuis et al., unpublished data).

V. FINAL REMARKS

This chapter began with a division of the immune system into an innate and a cognate immune system where recognition of an invading microorganism is nonspecific (and non-adaptive) or specific (and adaptive), the latter resulting in two types of immune responses leading to the production of effector/memory cells and/or molecules assisting the former in the eventual elimination of the intruder.

We have seen how the cognate immune system is intricately associated with the lymphoid system as a highly organized microenvironment instrumental as a "meeting place" between antigen and specific antigen reactive cells or immunologically competent cells derived from equally well-organized primary lymphoid organs or tissues like thymus and bone marrow.[86] Even without antigenic stimulation the histophysiology of the immune system is under neuroendocrine control, shaping the conditions in which, upon violation of the integrity of the body by an infectious agent, it can respond by a concerted action involving local cell-cell signaling between the cells involved and systemically thus giving off warning signals to the CNS. Subsequently, the CNS may interfere with the way effector cells are functioning, thereby superimposing its action over immune system-derived regulatory mechanisms. The idea of the immune system as a "sixth sense" for the CNS, which is thus capable of responding to stimuli otherwise going by unnoticed is a rather attractive one and is the subject of the remaining part of this book.

ENGLISH TRANSLATIONS

P. 6 "It is certain and beyond doubt that the lymph corpuscles are generated in the lymph glands, and essentially not from germs that the chyle brings into the node, but from those, that develop on the parenchyma of the node as on a motherly feeding layer."

P. 6 "Either this results from division of cells, which lie in the interstices of the reticulum of the nodules and strands; or it happens in this way that there is a continuous or intermittent extravasation of leukocytes from the blood vessels of the nodules and strands, so that there is a kind of continuous circulation of these elements out of the blood into the lymph and with the latter again into the blood."

P. 7 "The lymph nodes and the "intestinal follicles" are the hatcheries for the *de novo* formation of lymphocytes by way of indirect cell division".

(indirect cell division = normal mitosis in contrast to meiotic = direct division).

P. 7 "It will be difficult to doubt that it (the thymus), during its period of full development, serves the purpose of *de novo* formation of lymphocytes in the same way as later the lymph glands and lymphoid organs".

ACKNOWLEDGMENTS

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Heat Shock Protein 60 Is the Major Antigen Which Stimulates Delayed-Type Hypersensitivity Reaction in the Macaque Model of *Chlamydia trachomatis* Salpingitis

Anne B. Lichtenwalner,[1] Dorothy L. Patton,[1]* Wesley C. Van Voorhis,[2] Yvonne T. Cosgrove Sweeney,[1] and Cho-Chou Kuo[3]

Departments of Obstetrics and Gynecology,[1] Medicine and Pathobiology,[2] and Pathobiology,[3] University of Washington, Seattle, Washington

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Chlamydial delayed-type hypersensitivity antigens were analyzed by using the subcutaneous salpingeal autotransplant model of *Macaca nemestrina* infected with *Chlamydia trachomatis* serovar E. Heat shock protein 60 was the only antigen shown to induce delayed-type hypersensitivity among other antigens tested, including UV-inactivated organisms, recombinant major outer membrane protein, purified outer membrane proteins, and heat shock protein 10.

Chlamydia trachomatis infection continues to be one of the most important sexually transmitted diseases in the world and one of the most common sexually transmitted diseases in the United States. In the majority of cases, chlamydial infections are mild and self-limiting. However, in a subset of women, the extension of a cervical infection into the upper genital tract may induce pelvic inflammatory disease and salpingitis, which may lead to fallopian tube obstruction and infertility. The pathogenesis leading to tubal obstruction has not been defined. A genetic predisposition to pelvic inflammatory disease in women and in the macaque model has been noted (3, 9). However, clinical and animal studies have shown that the induction of a delayed-type hypersensitivity (DTH) reaction to *C. trachomatis* antigens leading to repeated and persistent infection of the upper reproductive tract plays an important role in tubal obstruction (10, 16). One of the chlamydial antigens which have been implicated in this immunopathogenesis is heat shock protein 60 (HSP60) (4, 13, 14, 17). However, whether other chlamydial antigens are involved in the immunopathogenesis of tubal obstruction has not been analyzed systematically. Therefore, in this study we utilized the subcutaneous salpingeal autotransplant ("pocket") macaque model to analyze whether any chlamydial antigens other than HSP60 may induce DTH. The pocket model allows us to test multiple antigens in a single animal.

(Part of this research was presented at Chlamydia 2002, Helsinki, Finland, 20 to 23 August 2002 [A. B. Lichtenwalner, D. L. Patton, W. C. Van Voorhis, Y. T. Cosgrove Sweeney, and C.-C. Kuo, Proc. 4th Meet. Eur. Soc. Chlamydia Res., p. 197-198].)

Sexually mature female pigtailed macaques (*Macaca nemestrina*) were used. Animals were housed at the University of Washington National Primate Research Center. The animal use protocol for this study was approved by the Animal Care Committee at the University of Washington.

* Corresponding author. Mailing address: Dorothy L. Patton, Department of Obstetrics and Gynecology, University of Washington, Box 356460, Seattle, WA 98195-6460. Phone: (206) 543-5554. Fax: (206) 616-9479. E-mail: dpatton@u.washington.edu.

The autotransplantation of salpingeal fimbrial tissue into subcutaneous abdominal "pockets" at multiple sites has been described previously (11, 12). Two weeks after the surgery, baseline histologic data were obtained by excising transplant tissue from each animal for histologic evaluation by standard hematoxylin and eosin staining. The remaining pockets were then inoculated with 10^5 inclusion-forming units of *C. trachomatis* serovar E in 50 μl of the chlamydial transport medium SPG, a phosphate buffer containing sucrose and glutamic acid (sucrose, 75 g; KH_2PO_4, 0.52 g; Na_2HPO_4, 1.22 g; glutamic acid, 0.72 g; H_2O to 1 liter [pH 7.4 to 7.6]). At 21 days postinoculation, two pockets were removed from each animal for the evaluation of baseline inflammatory reactions. One monkey was used for testing the dose response, and the remaining three monkeys were used for testing variously treated chlamydial antigens. The chlamydial antigens tested were UV-inactivated whole organisms, recombinant chlamydial HSP60 (rcHSP60), rcHSP10, major outer membrane protein (MOMP), and outer membrane complex protein (OMP). Control antigens used were recombinant glutathione *S*-transferase (rGST), which was used for cloning recombinant chlamydial antigens, and SPG. Fifty micrograms of antigen in 50 μl was inoculated into each pocket. Groups of two to six pockets per animal were randomly assigned to each test antigen. Pocket tissues were removed 48 h after inoculation for histologic examination. All surgical procedures were conducted while animals were under general anesthesia with ketamine and atropine.

The test antigens were prepared as follows. (i) To obtain purified chlamydial organisms, *C. trachomatis* serovar E (E/UW-5/Cx) elementary bodies (EBs) were grown in HeLa 229 cells and purified by density gradient centrifugation with diatrizoate meglumin (Hypaque-76; Winthrop-Breon Laboratories, New York, N.Y.) (6). (ii) To obtain inactivated organisms, chlamydial EBs were inactivated by UV irradiation (7). (iii) To obtain chlamydial outer membrane proteins, chlamydial proteins were fractionated into Sarkosyl-insoluble and -soluble fractions according to the method of Caldwell et al. (2). The Sarkosyl-insoluble fraction (MOMP) contains chlamydial outer membrane complexes, and the Sarkosyl-soluble fraction (OMP) contains the majority of the other chlamydial EB pro-

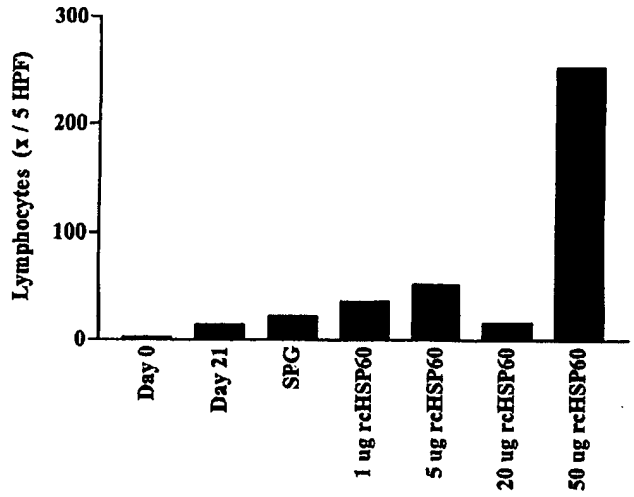

FIG. 1. Lymphocyte response to rcHSP60 at 48 h. Shown are lymphocyte counts at days 0 and 21 and at 48 h following challenge inoculation with the SPG control or increasing doses of rcHSP60 (one female macaque was tested; values are averages [x] for four pockets per test antigen). The response was predominantly lymphocytic and nonplasmocytic, typical of the DTH response to *Chlamydia* seen in this model. No dose responses were considered to occur in either plasma cells or neutrophils (data not presented). HPF, high-power fields.

teins, including HSP10 and HSP60. (iv) To obtain recombinant proteins, affinity-purified rcHSP60 (8) was obtained from R. Morrison and rcHSP10 was obtained from G. Bryne (8). rGST from *Schistosoma japonicum* was used to express rcHSP. All proteins were tested and found to be free of endotoxins (sensitivity, 0.1 endotoxin unit).

Swab samples were obtained for culture and ligase chain reaction for the detection of chlamydial infection from the removed tissues, which were subsequently placed in 10% formalin and processed for histologic examination. Fixed tissues were embedded in paraffin, thin sectioned, and stained with hematoxylin and eosin for light microscopic examination. T lymphocytes were identified by staining with a pan-T-cell stain, and polymorphonuclear leukocytes (were identified by a myeloperoxidase stain. Cells infiltrating the submucosa were counted in five randomly chosen high-power fields (×400 magnification), and the average number of cells was calculated. The observer was blind as to the tissue origins. Data were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple-comparison test. P values of 0.05 or less were considered significant.

Histologic examination of uninoculated pocket tissues showed no inflammation. A mild inflammation was observed 21 days after inoculation with live chlamydial organisms and before testing for DTH reactions (11). The dose-response experiment, involving a series of rcHSP60 concentrations (0, 5, 20, and 50 μg), showed a dose-dependent lymphocytic response (Fig. 1) characteristic of DTH at 48 h (11). The maximum reaction was observed at the 50-μg concentration. Tests with various antigens showed that only rcHSP60 elicited a significant lymphocytic reaction ($P < 0.05$, ANOVA and Tukey's multiple-comparison test) (Fig. 2). Mild lymphocytic reactions to MOMP, OMP, and rcHSP10 were also observed. However, the differences from controls were not statistically significant (Fig. 2). No plasma cell or polymorphonuclear leukocyte cell response was observed. No inflammatory reaction was observed in the tissues tested with control antigens. The OMP fraction contains chlamydial HSP60 (cHSP60) in addition to other proteins. In this protein fraction, the concentration of cHSP60 may not be high enough to induce a significant DTH reaction. The weak response of the whole EBs, which should contain cHSP60 in the chlamydial envelope, may be due to a proportionally smaller amount of cHSP60 relative to the total amount of protein injected into the pocket.

One of the limitations of this study was that the maximum systemic DTH response may not be induced by the infection of autotransplanted salpingeal tissues in the subcutaneous sites. Therefore, weak DTH reactions induced by testing with antigens other than cHSP60 may not be detected. Nevertheless, this study demonstrated that cHSP60 is the major antigen responsible for the DTH reaction in *C. trachomatis* salpingeal infection. Similar findings have been reported by Taylor et al. (15) for the ocular trachoma model in monkeys, for which these authors showed that formalin or UV-inactivated purified *C. trachomatis* EBs did not elicit DTH and neither did purified chlamydial MOMP or LPS. However, a soluble fraction of the triton extract of the organisms, known to contain cHSP60, did induce a DTH reaction.

The molecular mechanism of the DTH response to cHSP60 is of current interest. cHSP60 of *C. pneumoniae* has been shown to interact with Toll-like receptors, triggering innate immunity (1). Whether Toll-like receptors are involved in the immunopathogenesis of *C. trachomatis* genital tract infections in the pigtailed macaque has not been investigated. Such studies may further our understanding of immunopathogenesis in this model.

In conclusion, this study demonstrated that cHSP-60 does elicit DTH in the experimental salpingeal model used and

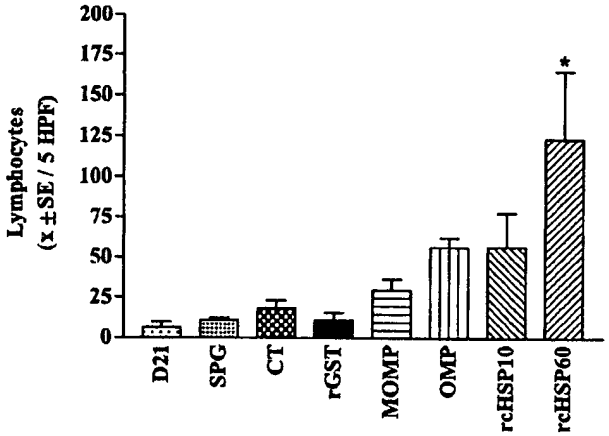

FIG. 2. Lymphocyte response at 48 h after challenge with various chlamydial proteins. Shown are lymphocyte counts at day 21 and at 48 h following challenge inoculation with SPG, killed chlamydiae (CT) or rGST (controls), or 50 μg of chlamydial antigens (three female macaques were tested; values are averages [x] for two to four pockets per treatment). *, $P \leq 0.05$ (ANOVA and Tukey's multiple-comparison test). Only for rcHSP60 was the lymphocyte response significantly different from those for the controls or for the chlamydial-protein preparations. HPF, high-power fields.

supports clinical observations indicating a role for cHSP60 in the immunopathogenesis of tubal damage (4, 17). This pathogenic mechanism is similar to that involved in ocular trachoma disease in experimental monkeys (5). This hypothesis should be further tested in an animal experiment to see whether animals sensitized with cHSP60 and infected with *C. trachomatis* in the salpinx develop severe salpingitis and tubal damage.

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During this study, animals were housed at the University of Washington National Primate Research Center. Prior approval for the use of these animals in this protocol was obtained from the Animal Care Committee at the University of Washington.

None of the authors have commercial or other associations that might pose a conflict of interest.

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Th1 Adjuvant *N*-Acetyl-D-Glucosamine Polymer Up-Regulates Th1 Immunity but Down-Regulates Th2 Immunity against a Mycobacterial Protein (MPB-59) in Interleukin-10-Knockout and Wild-Type Mice

YOSHIMI SHIBATA,[1]* IKURO HONDA,[2] J. PAUL JUSTICE,[1] MICHAEL R. VAN SCOTT,[1] REIKO M. NAKAMURA,[2] AND QUENTIN N. MYRVIK[3]

Department of Physiology, East Carolina University Brody School of Medicine, Greenville, North Carolina 27858[1]; Japan BCG Laboratory, Tokyo, Japan[2]; and Myrvik Enterprises, Southport, North Carolina 28461[3]

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Treatment of mice with heat-killed (HK) *Mycobacterium bovis* BCG or 1- to 10-μm chitin particles (nonantigenic *N*-acetyl-D-glucosamine polymers) is known to induce innate immune responses, including gamma interferon (IFN-γ) production, which plays a Th1 adjuvant role. However, HK BCG further induces prostaglandin E_2-releasing spleen macrophages (Mϕ) (PGE_2-Mϕ), which potentially inhibit Th1 adjuvant activities. We found that chitin particles did not induce PGE_2-Mϕ formation. To further assess whether chitin has Th1 adjuvant effects, interleukin-10 (IL-10)-knockout (KO) mice and their wild-type (WT, C57BL/6) controls were immunized with a 30-kDa MPB-59 mycobacterial protein mixed with chitin. Immunization with MPB-59 alone induced Th2 responses, characterized by increases in total serum immunoglobulin E (IgE) and specific serum IgG1 levels and spleen Th2 cells producing IL-4, IL-5, and IL-10. No IFN-γ-producing spleen Th1 cells, specific serum IgG2a, or delayed-type hypersentivity (DTH) footpad reactions were detected. On the other hand, chitin–MPB-59 immunization significantly increased spleen Th1 responses, DTH reaction, and serum IgG2a levels along with decreases of Th2 responses. The magnitude of these Th1 adjuvant effects was greater in IL-10-KO mice than in WT mice. In contrast, immunization with HK BCG–MPB-59 showed little or no Th1 adjuvant effect. These data indicate that chitin has a unique Th1 adjuvant effect on the development of Th1 immunity against a mycobacterial antigen. IL-10 down-regulates the adjuvant effect of chitin.

To develop protective immunity against intracellular infections such as tuberculosis, Th1 adjuvants play an important role. Live *Mycobacterium bovis* Calmette-Guerin bacillus (BCG) and Freund's complete adjuvant (FCA; heat-killed [HK] *M. tuberculosis* in mineral oil) have been used as Th1 adjuvants in experimental animals (15, 22, 52). Relatively high doses of HK BCG in saline compared with those of live BCG or FCA are required for the induction of nonspecific (innate) immune responses (26). However, HK BCG at high doses also induces prostaglandin E_2 (PGE_2)-releasing "suppressor" macrophages (Mϕ) (13, 30, 36). PGE_2 differentially modulates Th1 and Th2 immune responses. PGE_2 strongly inhibits the production of Th1 cytokines, such as interleukin-2 (IL-2), IL-12, and gamma interferon (IFN-γ), and, PGE_2, depending on stimulatory conditions, either has no effect or enhances production of the Th2-associated cytokines, such as IL-4, IL-5, and IL-10 (6, 16, 45, 47). Therefore, PGE_2-Mϕ appear to reduce Th1 adjuvant effects (14).

Recently, we have observed that Mϕ phagocytose HK BCG and HK *Propionibacterium parvum* (*Corynebacterium parvum*) through mannose receptors that recognize carbohydrates of cell walls, including *N*-acetyl-D-glucosamine, and produce Th1 cytokines, such as IL-12, IL-18, and tumor necrosis factor α (TNF-α) (38–40). To further study this mechanism, we have designed 1- to 10-μm *N*-acetyl-D-glucosamine polymer (chitin) particles that induce Mϕ to produce the cytokines at levels comparable to those stimulated by HK BCG or HK *C. parvum* (38, 39). However, unlike HK BCG or HK *C. parvum*, chitin particles do not induce PGE_2-Mϕ formation (this study). These observations suggest that chitin is a better Th1 adjuvant than HK BCG.

In this study, to determine Th1 adjuvant effects of chitin, we have examined whether soluble MPB-59 antigen mixed with chitin promotes Th1 immunity specific for MPB-59. MPB-59 is one of the 30-kDa mycobacterial antigens that are produced by proliferative BCG and *M. tuberculosis* and are predominant immunogens (21, 33, 35, 42, 49). When mice develop Th1 immunity against these antigens, they resist bacterial challenges (1, 20, 23, 32, 35). However, immunization with soluble MPB-59 alone resulted in typical Th2 responses including increases in specific serum immunoglobulin E (IgE) and splenic Th2 cells producing IL-4, IL-5, and IL-10. In this study, we present the results of the treatment with chitin as a Th1 adjuvant compared with those of the treatments with FCA or HK BCG suspended in saline.

Since it is established that endogenous IL-10 down-regulates various immune responses, including Th1 and Th2 responses (11, 18, 25, 28), we also employed IL-10-knockout (KO) mice, which were expected to provide a significantly higher magnitude of the chitin adjuvant effects.

MATERIALS AND METHODS

Mice. Breeding pairs of IL-10-KO (C57BL/6-Il10^{tm1Cgn}) mice (28) were obtained from the Jackson Laboratory (Bar Harbor, Maine). Offspring were raised under pathogen-free conditions. No mice used in this study showed colitis (39). Nonpregnant females, 8 to 14 weeks old, were used for experiments. Age-matched female C57BL/6 mice were obtained from the Jackson Laboratory and

* Corresponding author. Mailing address: Department of Physiology, East Carolina University Brody School of Medicine, Greenville, NC 27858. Phone: (252) 816-1905. Fax: (252) 816-3460. E-mail: shibatay@mail.ecu.edu.

used as wild-type (WT) control mice. Both IL-10-KO and WT mice were maintained in barrier-filtered cages and fed Purina laboratory chow and tap water ad libitum. Experimental protocols employed in this study were approved by IACUC of East Carolina University Brody School of Medicine.

Preparations of chitin particles and HK BCG. As described previously (38, 40), chitin particles (1 to 10 μm) were prepared from purified chitin powders (Sigma Chemical Co., St. Louis, Mo.), suspended in saline (20 mg/ml), autoclaved, and stored at 4°C until use. The cultured bacteria of *M. bovis* BCG Tokyo 172 strain (the Japanese vaccine) were washed, autoclaved, and lyophilized. The powder of HK BCG was suspended in saline immediately before use. The suspensions of both chitin and HK BCG were dispersed by brief sonication (10 s) prior to injection. These chitin and HK BCG preparations contained undetectable levels of endotoxin (<0.03 endotoxin units/ml), as determined by the *Limulus* amebocyte lysate assay (Sigma) (39). Similarly, HK *C. parvum* suspensions were prepared as previously described (36).

Purified MPB-59. MPB-59 (30 kDa) was prepared from culture filtrates of *M. bovis* BCG Tokyo 172 as described previously (19). The bacteria were cultured in Sauton synthetic medium at 37°C without aeration for 8 days. Sixty liters of culture filtrates was concentrated with ultrafiltration with a Pellicon Cassette system (XX42PEL60; Millipore, Bedford, Mass.) with a molecular weight 5,000 cutoff membrane (YM-3; Amicon, Beverly, Mass.). Proteins were further concentrated with 60% saturated ammonium sulfate and fractionated high-pressure liquid chromatography (i) affinity chromatography with phenyl Sepharose CL-4B, (ii) DEAE Sepharose CL-6B ion exchange, (iii) Sephacryl S200 HR gel filtration, and (iv) re-ion-exchange with DEAE Sepharose CL-6B (all from Pharmacia LKB, Uppsala, Sweden) (19). Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis with 10 μg of purified MPB-59 protein, a single 30-kDa band was stained by silver (data not shown). The procedure resulted in 4 mg of purified MPB-59 from 60 liters of culture filtrates.

Endotoxin removal. Endotoxin removal from all soluble materials for cultures and administration to mice were carried out by filtration and sterilization through 0.22-μm-pore-size Zetapore membranes (AMF-Cuno). The effectiveness of endotoxin removal was monitored by the *Limulus* amebocyte assay (Sigma).

Mouse immunization protocol and footpad DTH. Groups of mice (six/group) were given MPB-59 and/or chitin four times intraperitoneally at weekly intervals as follows: group I, MPB-59 (50 μg/dose) alone; group II, 1- to 10-μm chitin (200 μg/dose) alone; group III, mixtures of MPB-59 (50 μg/dose) and chitin (200 μg/dose); and group IV, saline (0.1 ml/dose) as controls. In some experiments, to determine whether HK BCG in saline at a dose that induces innate immune responses (Fig. 1B) has a Th1 adjuvant effect, we employed HK BCG (200 μg/dose) instead of chitin. Seven days after the final immunization, footpad delayed-type hypersensitivity (DTH) reactions to the locally injected MPB-59 were assessed. Mice received 50 μl of MPB-59 solution at 1,000 μg/ml in the right footpad and saline in the left footpad (control). After 48 h, mice were euthanized and MPB-59-induced footpad swelling was monitored with a spring-loaded metric caliper (Mitutoyo, Kawasaki, Japan). Spleens and blood were also harvested.

As a positive Th1 adjuvant control, 1 ml of saline with 500 μg of MPB-59 was mixed with 1 ml of FCA, and the mixture was given intraperitoneally to a group of mice (0.2 ml/dose) on days 0 and 14. Fourteen days after the final immunization, footpad DTH reactions were measured as described above.

Cytokine production in recall response—spleen cell cultures stimulated with MPB-59 antigen. Spleens in each group of mice were isolated and pooled. Spleen cells (4×10^6 cells/ml) were suspended in RPMI 1640 plus 10% fetal bovine serum and incubated with MPB-59 at 10, 20, and 50 μg/ml for 4 days. After the incubation, the culture supernatants were collected, and the levels of selected cytokines (IL-4, IL-5, IL-10, and IFN-γ) were measured by the appropriate specific enzyme-linked immunosorbent assay (ELISA) with commercially available reagents (PharMingen [San Diego, Calif.] and Endogen).

PGE_2-Mϕ. Plastic-adherent spleen Mϕ were prepared as described before (36, 37) and cultured in serum-free RPMI 1640 medium with or without calcium ionophore A23187 at 10^{-6} M for 2 h. PGE_2 levels in the culture supernatants were measured by a competitive ELISA (Cayman, Ann Arbor, Mich.).

Levels of IgE, IgG1, and IgG2a specific for MPB-59 in serum. Total serum IgE levels were detected by ELISA using purified mouse IgE κ isotype (PharMingen) as a standard and rat anti-mouse IgE monoclonal antibody, clone R35-72 (PharMingen), as a capture antibody. Levels of MPB-59-specific IgE, IgG1, and IgG2a were measured by ELISA with 96-well plates that were coated with MPB-59 at 0.3 μg/0.1 ml/well in 0.05 M sodium carbonate buffer, pH 9.6, overnight at 4°C. Biotinylated rat monoclonal antibodies detecting IgE, IgG1, and IgG2a were clones R35-92, A85-1, and R19-15, respectively (PharMingen).

Superoxide anion release assay. Superoxide anion levels released by alveolar Mϕ were measured by a cytochrome *c* reduction assay as described previously (38, 39). Plastic-adherent alveolar Mϕ were placed in HEPES-bicarbonate buffer containing 50 μM ferricytochrome *c* (Sigma) and incubated at 37°C for 1 h in the presence of phorbol myristate acetate (1 μM). The amount of reduced ferricytochrome *c* was measured by using a molecular extinction coefficient of 21.1 $mM^{-1}\ cm^{-1}$ from the change in absorbance at 550 nm against a cell-free blank. Superoxide formation was expressed as nanomoles per 10^6 cells.

Statistics. Data from this project were analyzed by one-way analysis of variance. For culture studies, tissues isolated from at least four mice were pooled; their cells were cultured in at least triplicate in each group. A *P* value of less than 0.05 is considered statistically significant.

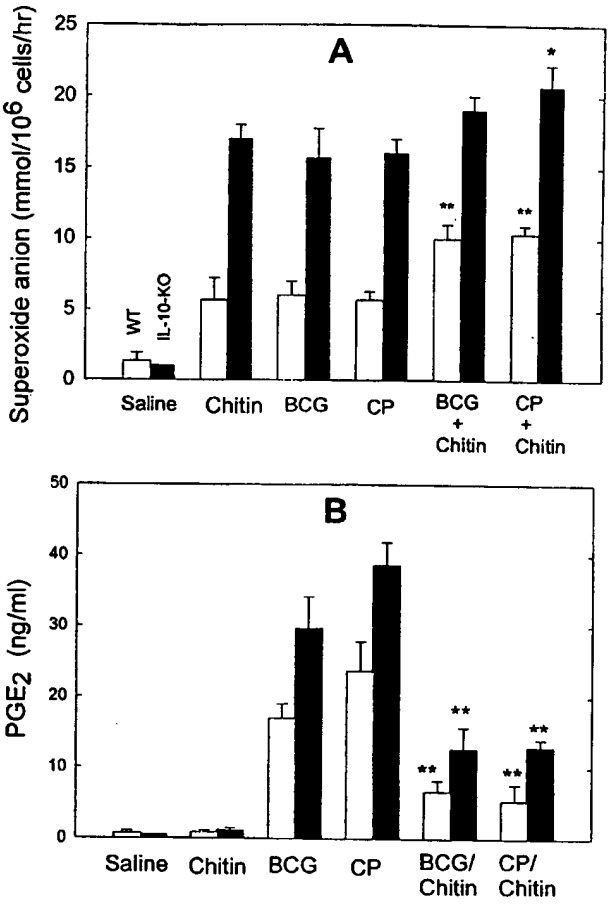

FIG. 1. Alveolar Mϕ priming and the formation of PGE_2-Mϕ in the spleen following HK BCG administration. WT and IL-10-KO mice intravenously received 0.5 mg of HK BCG, chitin, or HK *C. parvum* (CP; positive control). Mice that received 0.2 ml of saline served as negative controls. Furthermore, some groups received chitin (0.5 mg) mixed with HK BCG (0.5 mg) or HK *C. parvum* (0.5 mg). (A) Superoxide anion release by alveolar Mϕ. On day 3, alveolar Mϕ were assayed in vitro for superoxide anion release by phorbol myristate acetate (1 μM). Superoxide anion levels were measured by a cytochrome *c* reduction assay as described in Materials and Methods. Data are means plus standard deviation; $n = 4$. *, $P < 0.05$ compared with chitin alone; **, $P < 0.01$ compared with BCG alone or *C. parvum* alone. (B) PGE_2 release by spleen Mϕ. On day 7, splenic Mϕ were isolated from the other set of experimental groups. Mϕ in each group were pooled and incubated in serum-free RPMI 1640 medium containing A23187 at 10^{-6} M for 2 h. The levels of PGE_2 were measured by ELISA. Values are means plus standard deviations; $n = 3$. **, $P < 0.01$ compared with BCG alone or *C. parvum* alone.

RESULTS

Chitin induced alveolar Mϕ priming but not splenic PGE_2-Mϕ formation. Results comparable to those in Fig. 1A have

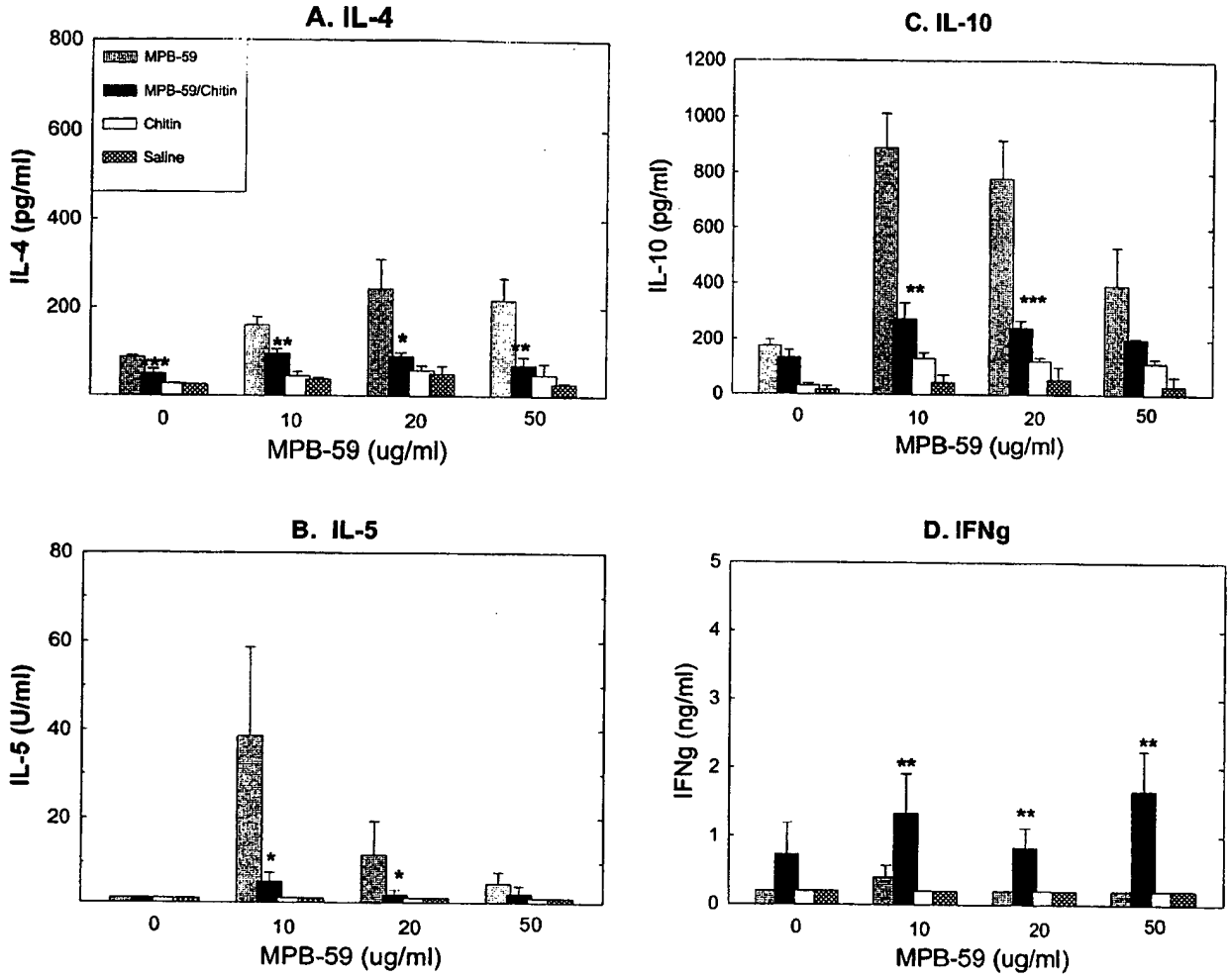

FIG. 2. Chitin-treated mouse spleen cells decreased MPB-59-stimulated IL-4, IL-5, and IL-10 production but increased MPB-59-stimulated IFN-γ production. Spleen cells were isolated from the WT mouse groups receiving the indicated treatment and stimulated in vitro with MPB-59 at 0 (medium), 10, 20, and 50 μg/ml for 4 days. The cytokine levels in the culture supernatants were measured by ELISA, as described in Materials and Methods. Values are means plus standard deviation from triplicate cultures. The data shown are representative of two independent experiments. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.005$ compared to the MPB-59-immunized group.

been reported earlier; the present observations are included because they validate assumptions necessary for interpretation of the present findings. Previous studies (38, 39) demonstrated that intravenous injection of bacteria or chitin results in the priming of alveolar Mϕ, involving the mechanisms of NK cell production of IFN-γ. To confirm whether HK BCG or chitin induces the priming of alveolar Mϕ, WT and IL-10-KO mice were given 0.5 mg of HK BCG, HK *C. parvum* (a positive control), or chitin intravenously. We isolated alveolar Mϕ from the groups and measured superoxide anion levels released by the Mϕ. We found that HK BCG, HK *C. parvum*, and chitin induced alveolar Mϕ priming at comparable levels on day 3 (Fig. 1A) but not on day 7 (data not shown). Furthermore, alveolar Mϕ on day 3 from mice receiving the chitin–HK-BCG or chitin–HK-*C. parvum* mixture slightly increased superoxide anion release (Fig. 1A). We also confirmed that endogenous IL-10 inhibited alveolar Mϕ priming levels (39).

To assess whether these treatments result in the formation of PGE_2-Mϕ in the spleen (36, 37), splenic Mϕ were isolated on day 7 and stimulated in vitro with A23187 at 10^{-6} M for 2 h. As shown in Fig. 1B, PGE_2 levels were unchanged in saline control and chitin-treated groups, whereas significantly higher levels of PGE_2 were observed in both HK-BCG- and HK-*C. parvum*-treated groups. IL-10-KO mice showed more PGE_2 than WT mice, suggesting that endogenous IL-10 inhibits splenic PGE_2-Mϕ formation. The PGE_2 production in vitro was over 90% inhibited by nimesulide, a PGG/H synthase-2 inhibitor, at 1 μM (data not shown). Interestingly, the group treated with the mixture of HK BCG with chitin (0.5 mg each) showed lower levels of PGE_2 than the group receiving HK BCG alone. As reported previously (36), splenic Mϕ on day 3, however, showed no detectable increase in PGE_2 levels in all groups (data not shown). Similar kinetics of PGE_2-Mϕ formation were observed when HK BCG or HK *C. parvum* was given intraperitoneally and subcutaneously (data not shown).

Recall responses of spleen cell cultures from mice coimmunized with MPB-59 and chitin. To determine whether MPB-59-induced Th2 cell development was modulated by coinjected

chitin, selected cytokine levels produced by Th1 and Th2 cells were measured in recall responses of spleen cell cultures. When spleen cells were prepared from MPB-59-immunized WT mice and stimulated in vitro by MPB-59 at 10, 20, and 50 μg/ml, relatively large amounts of IL-4, IL-5, and IL-10, but not IFN-γ, were detected (Fig. 2). When mice were coimmunized with chitin and MPB-59, the levels of IL-4, IL-5, and IL-10 were significantly reduced (Fig. 2A to C). In contrast, IFN-γ production was significantly increased (Fig. 2D). However, there was little or no production of these cytokines when spleen cells were prepared from saline- or chitin-treated WT control mice and stimulated in vitro by MPB-59 antigen (Fig. 2).

To determine whether endogenous IL-10, which is produced by diverse cell populations, including antigen-stimulated Th2 cells (28), down-regulates Th1 or Th2 responses, we immunized IL-10-KO mice with MPB-59 mixed with chitin. As shown in Fig. 3, IL-4 and IL-5 production was higher in the recall responses of MPB-59-immunized IL-10-KO mice than in those of MPB-59-immunized WT mice (Fig. 2A and B). When IL-10-KO mice were coimmunized with MPB-59–chitin, higher levels of IFN-γ were observed along with marked reduction of IL-4 and IL-5 production (Fig. 3). The results support the previous observations that IL-10 down-regulated both antigen-specific Th1 and Th2 responses (11, 18, 25, 29).

Serum IgG1, IgG2a, and IgE levels in mice coimmunized with MPB-59 and chitin. We observed that immunization of WT mice with MPB-59 resulted in increases in levels of total IgE and MPB-59-specific IgG1 in serum (Fig. 4A and C). Since endogenous IL-4 and IFN-γ isotype-switching signals antigen-specific B cells, which bias the serum IgE and IgG1 and the serum IgG2a, respectively (8, 44), we determined if these heavy-chain class switches are developed by coimmunization of MPB-59 and chitin. As shown in Fig. 4D, there was a relatively low level of serum IgG2a. In contrast, after coimmunization with MPB-59 and chitin, the levels of IgG1 and IgE were significantly reduced (Fig. 4A and C). Interestingly, MPB-59-immunized IL-10-KO mice showed a significant enhancement of total IgE, MPB-59-specific IgE, and MPB-59-specific IgG1 levels compared with those in WT mice; following immunization with MPB-59 and chitin, IgG2a levels were also significantly enhanced (Fig. 4).

Our results suggest that MPB-59 is a strong allergen which induces IL-4-dependent IgG1 and IgE production (8). Chitin-induced endogenous IFN-γ appears to regulate antibody heavy-chain class switching, resulting in higher IgG2a levels (44). Furthermore, endogenous IL-10 appears to down-regulate IL-4-dependent IgG1 and IgE production and IFN-γ-dependent IgG2a production.

Footpad DTH reaction in mice coimmunized with MPB-59 and chitin. To determine if chitin has adjuvant effects to develop DTH reactions, mice were immunized with MPB-59 mixed with chitin. As shown in Fig. 5, 2 days after the challenge with MPB-59 in the footpad, the thickness of the footpads was measured. Both WT and IL-10-KO mice showed significant footpad thickness following the challenge. Although the footpad reactions seemed to be stronger in IL-10-KO than in WT mice, there was no statistically significance between MPB-59–chitin-immunized IL-10-KO and WT mice (Fig. 5).

Does coinjected HK BCG provide a Th1 adjuvant effect? To determine whether HK BCG has a Th1 adjuvant effect,

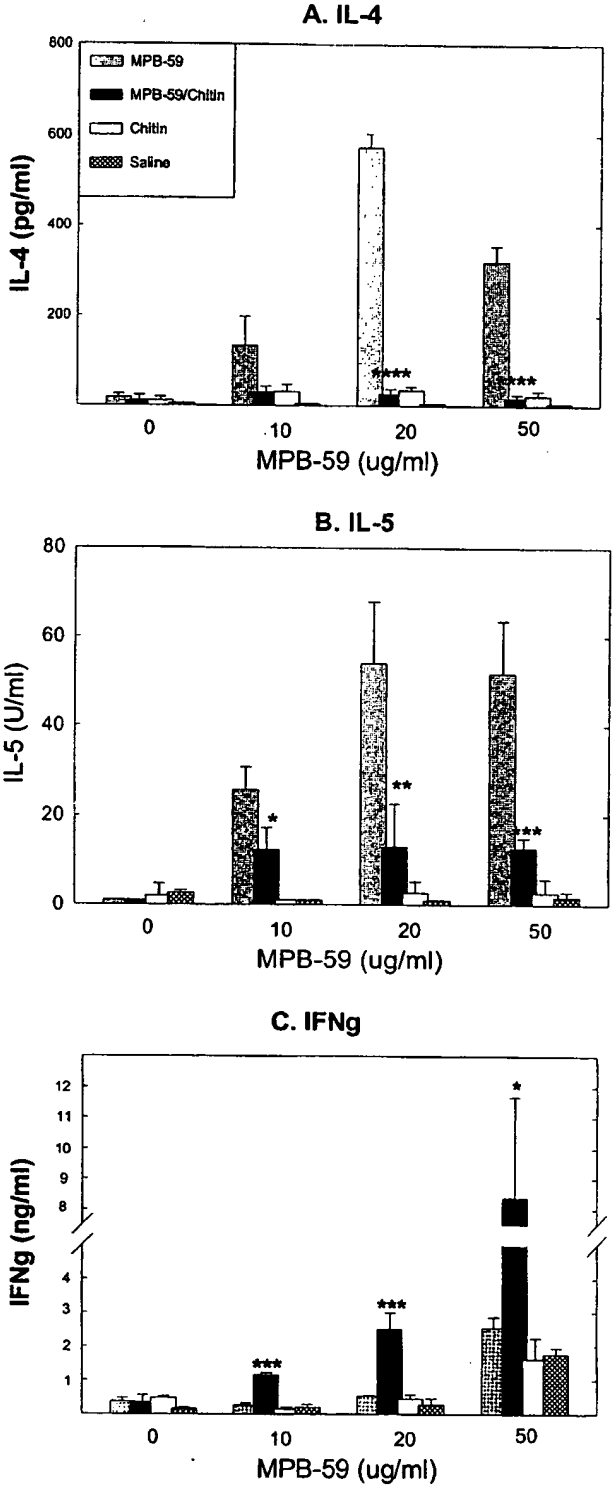

FIG. 3. Chitin-treated mouse spleen cells decreased MPB-59-stimulated IL-4 and IL-5 production but increased MPB-59-stimulated IFN-γ production in IL-10-KO mice. IL-10-KO mice were immunized as described in Materials and Methods. Recall responses of spleen cell cultures were assayed as described in the Fig. 1 legend. Values are means plus standard deviations from triplicate cultures. The data shown are representative of two independent experiments. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.005$ compared to the MPB-59-immunized group.

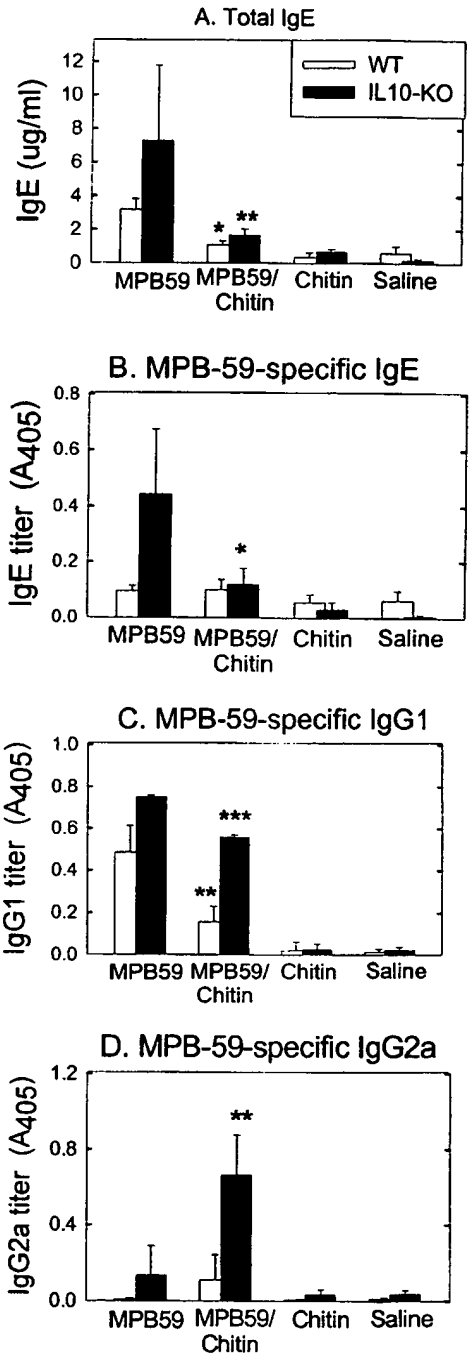

FIG. 4. Chitin treatment modulated total IgE levels and MPB-59-specific-antibody formation (IgE, IgG1, and IgG2a) in WT and IL-10-KO mice. Sera were isolated from WT and IL-10-KO mice that were immunized with MPB-59, MPB-59–chitin, chitin, and saline as described in Materials and Methods. (A) Total IgE levels in the sera were measured by a sandwich ELISA. Values are means plus standard deviations; $n = 6$. (B through D) MPB-59-specific IgE, IgG1, and IgG2a in sera were quantitated as described in Materials and Methods. The sera were diluted 1/5, 1/100, and 1/20 with saline before they were assayed for MPB-59-specific IgE, IgG1, and IgG2a levels, respectively. Values are mean plus standard deviations; $n = 6$. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.005$ compared to the MPB-59-immunized group.

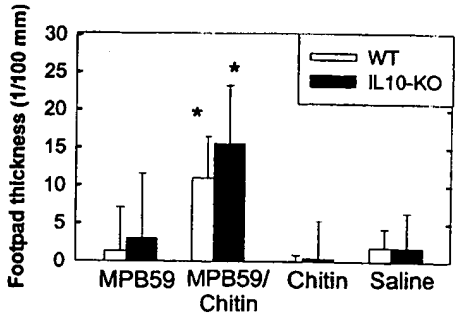

FIG. 5. Development of MPB-59-induced footpad DTH in WT and IL-10-KO mice coimmunized with MPB-59 and chitin. WT and IL-10-KO mice were immunized with MPB-59, MPB-59–chitin, chitin, and saline as described in Materials and Methods. Seven days after the final immunization, mice received 50 μg of MPB-59 solution in the right footpad and 50 μl of saline in the left footpad (control). After 48 h, right footpad thickness minus left footpad thickness in each group of mice was calculated. Values are means plus standard deviations; $n = 6$. * and **, $P < 0.05$ and $P < 0.01$ compared to the MPB-59-immunized group.

C57BL/6 (WT) mice (six per group) were immunized with MPB-59 mixed with HK BCG (200 μg/dose) in saline at schedules and in groups similar to those receiving coinjected chitin. As a positive Th1 adjuvant, additional mice were immunized with MPB-59 mixed with FCA.

Figure 6 summarizes the IL-4 and IFN-γ levels in recall responses of spleen cell cultures, MPB-59-specific serum IgE and IgG2a, and footpad DTH reactions. MPB-59 in FCA enhanced footpad DTH reactions and antigen-specific IgG2a levels and reduced IgE levels. This Th2-to-Th1 shift was associated with relatively high IFN-γ levels and low IL-4 levels in recall responses. In contrast, mice immunized with MPB-59 mixed with HK BCG in saline showed neither up-regulation of Th1 responses nor down-regulation of Th2 responses specific for MPB-59.

DISCUSSION

Previously, we observed that phagocytosable nonantigenic chitin, a seemingly inert molecule, as well as HK BCG and HK *C. parvum*, induced endogenous Th1 cytokines (IL-12, IL-18, TNF-α, and IFN-γ) (38–40). These cytokines are generally seen at early stages of infection (innate immunity) caused by mycobacteria and other intracellular bacteria (39). Innate immunity is important for protection against intracellular bacterial infections and to induce Th1 responses and cell-mediated immunity against bacteria (2). It is well established that Th1 cytokines down-regulate allergic immune (Th2) responses (34). Consistent with our previous study (41), the present study clearly demonstrated that chitin, as a Th1 adjuvant, down-regulates antigen-specific Th2 responses and up-regulates Th1 responses specific for a mycobacterial antigen.

The provocative findings are that MPB-59 induces Th2-dominant immune responses, including those of IL-4-, IL-5-, and IL-10-producing splenic Th2 cells, and increases in total serum IgE and MPB-59-specific IgG1 levels. Increases in these inflammatory parameters have been demonstrated in typical airway allergic responses (41). In this study, we found that MPB-59 immunization did not establish DTH reactions. In

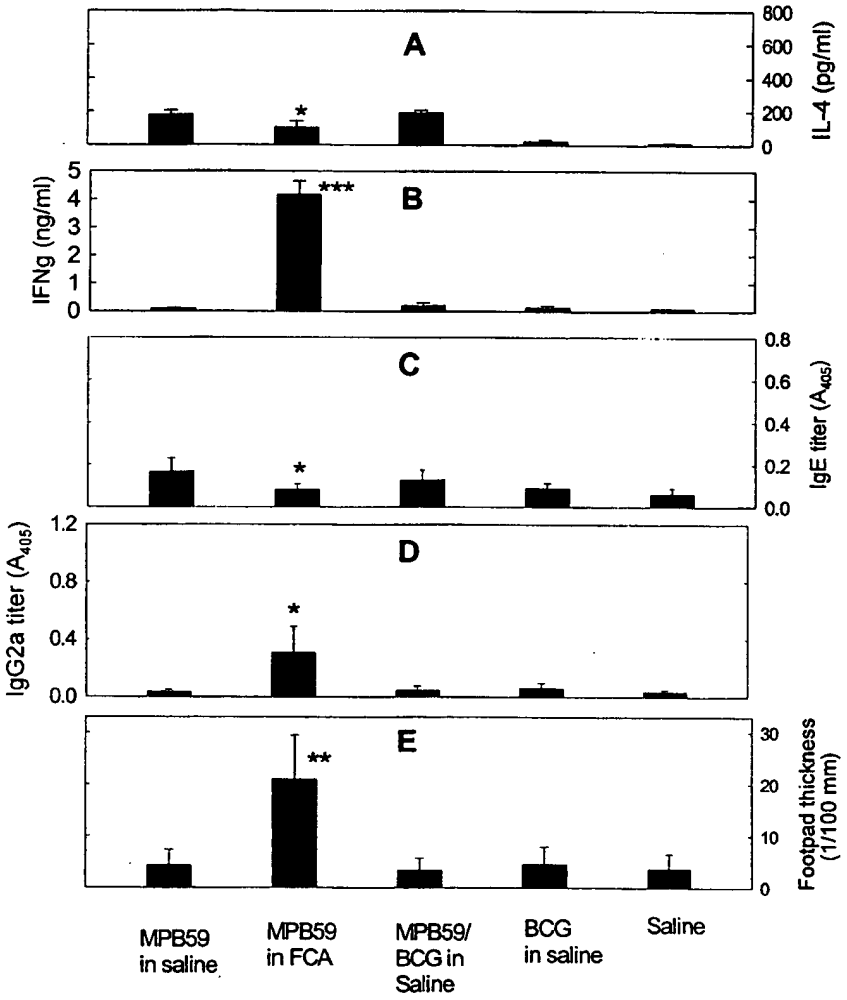

FIG. 6. Immunity against MPB-59 when mice were immunized with MPB-59 mixed with HK BCG in saline or mixed with FCA. C57BL/6 mice were immunized with MPB-59, MPB-59 in FCA, MPB-59 mixed with HK BCG in saline, HK BCG, and saline as described in Materials and Methods. (A and B) IL-4 and IFN-γ levels produced in recall responses of spleen cell cultures, respectively. Spleen cells were isolated from each group of mice stimulated in vitro with MPB-59 at 20 μg/ml for 4 days. The levels of IL-4 and IFN-γ in the culture supernatants were measured by ELISA, as described in Materials and Methods. Values are means plus standard deviations from triplicate cultures. (C and D) MPB-59-specific IgE and IgG2a titers, respectively, in serum. Immediately after footpad thickness measurements, sera were isolated from all groups of mice. MPB-59-specific IgE and IgG2a in sera were quantitated as described in Materials and Methods. Values are means plus standard deviations; n = 6. (E) DTH reaction (footpad thickness). Seven days after the final immunization (14 days after the second injection of MPB-59 in FCA), mice received 50 μg of MPB-59 solution in right footpad and 50 μl of saline in left footpad (control). After 48 h, right footpad thickness minus left footpad thickness in each group of mice was calculated. Values are means plus standard deviations; n = 6. The data are representative of two independent experiments. *, **, and ***, $P < 0.05$; $P < 0.005$, and $P < 0.0005$ compared to the MPB-59-immunized group.

contrast, when mice were immunized with MPB-59 mixed with chitin, chitin down-regulated these Th2-dominant responses and up-regulated IFN-γ-producing Th1 cells. This increase in IFN-γ levels is associated with an increase in MPB-59-specific IgG2a levels that illustrates isotype switching by B cells (44). Under these Th1-dominant conditions, MPB-59 induces local DTH responses. It has been reported that DTH is IFN-γ dependent but requires additional factors such as IL-8, TNF-α, and migration inhibitory factor produced by Mϕ and activated T cells (5, 9).

It is particularly important that HK BCG at a dose that induced innate immune responses including IFN-γ production did not down-regulate Th2 responses or up-regulate Th1 responses in the MPB-59 immunization model (Fig. 6). Previous studies showed that BCG immunotherapies in cancer induce suppressor T cells and suppressor Mϕ (3, 13, 30) that reduce protective immunity against tuberculosis and cancer. Recent studies suggest that suppressor T cell functions can be, at least in part, explained by development of mycobacterium-specific Th2 cells (25, 46, 51, 54). Suppressor Mϕ that release PGE_2 would be associated with this shift of Th1-to-Th2 response (14, 16, 45, 47). It is of particular importance that effective Th1 adjuvants should not induce but inhibit the formation of PGE_2-Mϕ (14), although the mechanisms of chitin treatments that inhibit PGE_2-Mϕ formation (Fig. 1) remain to be elucidated.

It should be noted that HK BCG in light mineral oil, HK *Listeria monocytogenes* in Freund's incomplete adjuvant, and HK *M. tuberculosis* in mineral oil (FCA) have been used ex-

tensively for the enhancement of cell-mediated immunity against coinjected antigens (17, 53). The present study showed that FCA induces Th1 responses specific for coinjected MPB-59 (Fig. 6). However, cell walls isolated from BCG, *M. tuberculosis*, and *C. parvum* appear to contain essential components for the induction of splenic PGE_2-Mϕ formation (13). FCA at the dose used in this study (0.01 mg of HK *M. tuberculosis*/dose) did not induce PGE_2-Mϕ, while HK BCG at ≥0.1 mg/dose in either saline or mineral oil induced PGE_2-Mϕ (13). Therefore, the adjuvant effects of HK BCG at various concentrations suspended in mineral oil or in saline remain to be elucidated (26, 47).

Observations in our earlier (39) and present studies showed that antigen-stimulated Th2 cells, chitin-stimulated Mϕ, and HK-BCG-stimulated Mϕ produce IL-10. In addition, many other diverse cell populations, including bronchial epithelial cells and B cells, produce IL-10 (7, 29). Endogenous IL-10 is a powerful negative regulator for chitin- or HK-BCG-induced innate immune responses characterized by the production of IL-12, IL-18, TNF-α, and IFN-γ (39). IL-10 also inhibits protective immunity against intracellular bacterial infections due to the down-regulation of IFN-γ production (4, 11, 12). It has also been reported that IL-10 inhibits Th2 responses to allergens, most likely by inhibiting antigen-presenting cells (11, 18, 25, 39). The present study confirms that immunization of IL-10-KO mice with MPB-59 induces significantly higher levels of serum IgE- and IgG1-producing and IL-4- and IL-5-producing Th2 cells than MPB-59 immunization of WT controls. Furthermore, chitin as a Th1 adjuvant induces MPB-59-specific Th-1 cells, footpad DTH, and serum IgG2a in IL-10-KO mice. Our studies clearly support the conclusion that endogenous IL-10 down-regulates the development of antigen-specific Th1 and Th2 responses rather than inducing the shift of Th1 to Th2 responses.

It has been established that several other bacteria and their components (24, 31, 40, 43, 48, 50, 53), such as lipopolysaccharide, superantigens, and DNA with unmethylated CpG motifs, induce Th1 cytokines that up-regulate Th1 responses with down-regulation of Th2 responses. Their efficacy in regulating immune responses is limited by some toxic side effects, including splenomegaly (10, 27) as well as the formation of PGE_2-Mϕ in the spleen. The chitin treatments in this study accomplished significant modification without any visible adverse effects, splenomegaly (data not shown), or splenic PGE_2-Mϕ formation. As a result, chitin preparations of nonmicrobial origin represent a very attractive new class of Th1 adjuvant.

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trinucleotide repeats in a causative gene (e.g., fragile X syndrome, myotonic dystrophy, Huntington disease).

an·ti·cli·nal (an-tē-klī'năl). Inclined in opposite directions, as two sides of a pyramid. [anti- + G. *klinō*, to incline]

an·tic·ne·mi·on (an-tik-nē'mē-on). SYN anterior border of tibia. [G. *antiknēmion*]

an·ti·co·ag·u·lant (an'tē-kō-ag'ū-lant). **1.** Preventing coagulation. **2.** An agent having such action (e.g., warfarin).

lupus a., antiphospholipid antibody causing elevation in partial thromboplastin time; associated with venous and arterial thrombosis.

an·ti·co·don (an-tē-kō'don). The trinucleotide sequence complementary to a codon found in one loop of a tRNA molecule; e.g., if a codon is A–G–C, its anticodon is U (or T)–C–G. The complementarity principle arises from Watson-Crick base-pairing, in which A is complementary to U (or T) and G is complementary to C. Sometimes called "nodoc."

an·ti·com·ple·ment (an-tē-kom'plĕ-ment). A substance that combines with a complement component and neutralizes its action by preventing its union with an antibody. SYN antialexin.

an·ti·com·ple·men·ta·ry (an'tē-kom-plĕ-men'tă-rē). Denoting a substance possessing the power of diminishing or abolishing the action of a complement.

an·ti·con·ta·gious (an'tē-kon-tā'jŭs). Preventing contagion.

an·ti·con·vul·sant (an'tē-kon-vŭl'sant). **1.** Preventing or arresting seizures. **2.** An agent having such action. SYN anticonvulsive, antiepileptic.

an·ti·con·vul·sive (an'tē-kon-vŭl'siv). SYN anticonvulsant.

an·ti·cu·ra·re (an-tē-koo-rä'-rē). A drug property referring to the capacity to reverse the muscle paralysis produced by *d*-tubocurarine and other curarelike neuromuscular blocking drugs. Examples include neostigmine, pyridostigmine, and edrophonium.

an·ti·cus (an-tī'kŭs). A term in anatomic nomenclature to designate a muscle or other structure which of all similar structures is nearest the front or ventral surface. Nomina Anatomica uses "anterior" in place of this term. [L. in the very front, fr. *ante*, before]

an·ti·cy·to·tox·in (an'tē-sī-tō-tok'sin). A specific antibody that inhibits or destroys the activity of a cytotoxin.

an·ti·de·pres·sant (an'tē-dē-pres'ănt). **1.** Counteracting depression. **2.** An agent used in treating depression.

tetracyclic a., a class of a.'s similar to the tricyclic a.'s and also related to the phenothiazine antipsychotics; e.g., maprotiline.

triazolopyridine a., a class of a.'s structurally and pharmacologically unrelated to other a.'s; clinical effectiveness appears to be equivalent to the tricyclic a.'s, but with less anticholinergic side effects; e.g., trazodone.

tricyclic a., a chemical group of a. drugs that share a 3-ringed nucleus; e.g., amitriptyline, imipramine, desipramine, and nortriptyline.

an·ti·di·a·bet·ic (an'tē-dī-ă-bet'ik). Counteracting diabetes; denoting an agent that lowers blood sugar (e.g., tolbutamide, insulin).

an·ti·di·ar·rhe·al, an·ti·di·ar·rhet·ic (an'tē-dī-ă-re'ăl, -dī-ă-ret'ik). **1.** Having the property of opposing or correcting diarrhea. **2.** An agent having such action (e.g., loperamide).

an·ti·di·u·re·sis (an'tē-dī-ū-rē'sis). Reduction of urinary volume.

an·ti·di·u·ret·ic (an'tē-dī-ū-ret'ik). An agent that reduces the output of urine.

an·ti·dot·al (an-tē-dō'tăl). Relating to or acting as an antidote.

an·ti·dote (an'tē-dōt). An agent that neutralizes a poison or counteracts its effects. [G. *antidotos*, fr. *anti*, against, + *dotos*, what is given, fr. *didōmi*, to give]

chemical a., a substance that unites with a poison to form an innocuous chemical compound.

mechanical a., a substance that prevents the absorption of a poison.

physiologic a., an agent that produces systemic effects contrary to those of a given poison.

universal a., a dated mixture of 2 parts activated charcoal, 1 part tannic acid, and 1 part magnesium oxide intended to be administered to patients who consumed poison. The mixture is ineffective and no longer used; activated charcoal is useful.

an·ti·drom·ic (an-tē-drom'ik). Denoting the propagation of an impulse along a conduction system (e.g., nerve fiber) in the direction opposite to which it normally travels.

an·ti·dys·en·ter·ic (an'tē-dis-en-ter'ik). Relieving or preventing dysentery.

an·ti·dys·rhyth·mic (an'tē-dis-rith'mik). SYN antiarrhythmic.

an·ti·dys·u·ric (an'tē-dis-ū'rik). Preventing or relieving strangury or distress in urination.

an·ti·e·met·ic (an'tē-ĕ-met'ik). **1.** Preventing or arresting vomiting. **2.** A remedy that tends to control nausea and vomiting. [anti- + G. *emetikos*, emetic]

an·ti·e·ner·gic (an'tē-en-er'jik). Acting against or in opposition. [anti- + G. *energos*, active]

an·ti·en·zyme (an-tē-en'zīm). An agent or principle that retards, inhibits, or destroys the activity of an enzyme; may be an inhibitory enzyme or an antibody to an enzyme (e.g., serum antitrypsin).

an·ti·ep·i·lep·tic (an'tē-ep-i-lep'tik). SYN anticonvulsant.

an·ti·es·tro·gen (an'tē-es'trō-jen). Any substance capable of preventing full expression of the biological effects of estrogenic hormones on responsive tissues, either by producing antagonistic effects on the target tissue, as androgens and progestogens do, or by competing with estrogens at estrogen receptors at the cellular level (e.g., tamoxifen).

an·ti·fe·brile (an-tē-fē'brīl, -feb'ril). SYN antipyretic (1). [anti- + L. *febris*, fever]

an·ti·fi·bril·la·tory (an'tē-fī'bri-lă-tōr-ē). Any measure or medication that tends to suppress fibrillary arrhythmias (atrial fibrillation, ventricular fibrillation).

an·ti·fi·bri·nol·y·sin (an'tē-fī-bri-nol'i-sin). SYN antiplasmin.

an·ti·fi·bri·no·lyt·ic (an'tē-fī-brin-ō-lit'ik). Denoting a substance that decreases the breakdown of fibrin; e.g., aminocaproic acid.

an·ti·fo·lic (an-tē-fō'lik). **1.** Antagonistic to the action of folic acid. **2.** Any agent with this effect. SEE ALSO folic acid *antagonists*, under *antagonist*.

an·ti·fun·gal (an-tē-fŭng'ăl). SYN antimycotic.

an·ti-G. In the strict sense, a term that means "antigravity" but, as commonly used, an adjectival term that implies protection against the effects of gravity (e.g., anti-G *suit*).

ANTIGEN

an·ti·gen (Ag) (an'ti-jen). Any substance that, as a result of coming in contact with appropriate cells, induces a state of sensitivity and/or immune responsiveness after a latent period (days to weeks) and that reacts in a demonstrable way with antibodies and/or immune cells of the sensitized subject in vivo or in vitro. Modern usage tends to retain the broad meaning of a., employing the terms "antigenic determinant" or "determinant group" for the particular chemical group of a molecule that confers antigenic specificity. SEE ALSO hapten. SYN immunogen. [anti(body) + G. *-gen*, producing]

ABO a.'s, see ABO blood group, Blood Groups appendix.

acetone-insoluble a., SYN cardiolipin.

allogeneic a. (al'ō-jĕ-ne'ik), genetic variations of the same a.'s within a given species.

Am a.'s, allotypic determinants (antigens) on the heavy chain of human IgA molecules.

Au a., (1) see Auberger blood group, Blood Groups appendix; **(2)** SYN Australia a.

Aus a., SYN Australia a.

Australia a., so-called because it was first recognized in an Australian aborigine, but now known to be subunits of the hepatitis B virus surface antigen. SYN Au a. (2), Aus a.

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after a series of previously applied stimuli. [G. *a-* priv. + *dia,* through, + *phoros,* bearing]

adi·a·spi·ro·my·co·sis (ă'dē-ă-spī'rō-mī-kō'sis). A rare pulmonary mycosis of humans and of rodents and other animals that dig in soil or are aquatic, caused by the fungus *Emmonsia parva var. crescens.*

adi·a·spore (a'dē-ă-spōr). A fungus spore which, when growing in the lungs of an animal or incubated in vitro at elevated temperatures, increases greatly in size without eventual reproduction or replication. [G. *a-* priv. + *dia,* through, + *sporos,* seed]

adi·as·to·le (ă-dī-as'tō-lē). Absence or imperceptibility of the diastolic movement of the heart; diastolic ventricular functional abnormality. Mostly European usage. [G. *a-* priv. + *diastolē,* dilation]

adi·a·ther·man·cy (ă-dī-ă-ther'man-sē). Impermeability to heat. [G. *dia-thermainō,* to warm through, fr. *a-* priv. + *dia,* through, + *thermē,* heat]

Adie, William J., Australian physician, 1886–1935. SEE A. *pupil, syndrome;* Holmes-Adie *pupil;* Holmes-Adie *syndrome.*

ad·i·em·or·rhy·sis (ad'i-em-ōr'i-sis). Arrest of the capillary circulation. [G. *a-* priv. + *dia,* through, + *haima,* blood, + *rhysis,* a flowing]

Adin·i·da (ă-din'i-dă). A suborder of dinoflagellates, in which the flagella are free and do not lie in furrows. [G. *a-* priv. + *diēn,* a whirling]

adip-, adipo-. Fat, fatty. Corresponds to G. lip-, lipo-. SEE ALSO lipo-. [L. *adeps, adipis,* soft animal fat, lard, grease; fatty tissue; obesity; akin to G. *aleipha,* unguent, anointing-oil, oil, fat, pitch, resin; *lipos,* animal fat, lard, tallow, vegetable oil]

adiph·e·nine hy·dro·chlo·ride (ă-dif'ĕ-nen). A spasmolytic agent used to decrease spasm of the biliary tract, gastrointestinal tract, uterus, and ureter.

adip·ic ac·id (ă-dip'ik). Hexanedioic acid; the dicarboxylic acid, $HOOC(CH_2)_4COOH$.

Ad·i·pi·o·done. SYN iodipamide.

adipo-. SEE adip-.

ad·i·po·cel·lu·lar (ad'i-pō-sel'ū-lăr). Relating to both fatty and cellular tissues, or to connective tissue with many fat cells.

ad·i·po·cer·a·tous (ad-i-pō-ser'ă-tŭs). Relating to adipocere. SYN lipoceratous.

ad·i·po·cere (ad'i-pō-sēr). A fatty substance of waxy consistency into which dead animal tissues (as those of a corpse) are sometimes converted when kept from the air under certain favoring conditions of temperature. SYN grave wax, lipocere. [adipo- + L. *cera,* wax]

ad·i·po·cyte (ad'i-pō-sīt). SYN fat *cell.*

ad·i·po·gen·e·sis (ad'i-pō-jen'ĕ-sis). SYN lipogenesis.

ad·i·po·gen·ic, ad·i·pog·e·nous (ad'i-pō-jen'ik, ad-i-poj'ĕ-nŭs). SYN lipogenic.

ad·i·poid (ad'i-poyd). SYN lipoid. [adipo- + G. *eidos,* resemblance]

ad·i·po·ki·net·ic (ad'i-pō-ki-net'ik). Denoting a substance or factor that causes mobilization of stored lipid. [adipo- + G. *kinēsis,* movement]

ad·i·po·ki·nin (ad-i-pō-kī'nin). An anterior pituitary hormone that causes mobilization of fat from adipose tissue. SYN adipokinetic hormone.

ad·i·pom·e·ter (ad-i-pom'ĕ-ter). An instrument for determining the thickness of the skin. [adipo- + G. *metron,* measure]

ad·i·po·ne·cro·sis (ad'i-pō-ne-krō'sis). Rarely used term referring to necrosis of fat, as in hemorrhagic pancreatitis.

ad·i·po·sal·gia (ad'i-pō-sal'jē-ă). Condition in which painful areas of subcutaneous fat develop. [adipo- + G. *algos,* pain]

ad·i·pose (ad'i-pōs). Denoting fat.

ad·i·po·sis (ad-i-pō'sis). Excessive local or general accumulation of fat in the body. SYN lipomatosis, liposis (1), steatosis (1). [adipo- + G. *-osis,* condition]

a. cerebra'lis, obesity resulting from intracranial disease, most commonly of the hypothalamus, resulting in hyperphagia.

a. doloro'sa, a condition characterized by a deposit of symmetrical nodular or pendulous masses of fat in various regions of the body, with discomfort or pain. SYN Anders disease, Dercum disease, lipomatosis neurotica.

a. or'chica, SYN adiposogenital *dystrophy.*

a. tubero'sa sim'plex, a condition resembling a. dolorosa, in which the fat occurs in small, nodular masses, which are sensitive to touch and may be spontaneously painful, on the abdomen or on the extremities.

a. universa'lis, excessive deposition of fat throughout all parts of the body, including the viscera.

ad·i·pos·i·ty (ad-i-pos'i-tē). **1.** SYN obesity. **2.** Excessive accumulation of lipids in a site or organ.

ad·i·po·su·ria (ad'i-pō-soo'rē-ă). SYN lipuria. [adipo- + G. *ouron,* urine]

adip·sia, adip·sy (ă-dip'sē-ă, -dip'sē). Absence of thirst or the lack of desire to drink. [G. *a-* priv. + *dipsa,* thirst]

ad·i·tus (ad'i-tŭs) [TA]. SYN aperture, inlet. [L. access, fr. *ad-eo,* pp. *-itus,* go to]

a. ad an'trum [TA], SYN a. to mastoid antrum.

a. ad antrum mastoideum [TA], SYN a. to mastoid antrum.

a. ad aqueduc'tum cer'ebri, SYN *opening* of aqueduct of midbrain.

a. ad infundib'ulum [TA], SYN infundibular *recess.*

a. ad sac'cum peritone'i mino'rem, SYN omental *foramen.*

a. glot'tidis infe'rior, SYN infraglottic *cavity.*

a. glot'tidis supe'rior, SYN intermediate laryngeal *cavity.*

laryngeal a. [TA], SYN laryngeal *inlet.*

a. laryn'gis [TA], SYN laryngeal *inlet.*

a. to mastoid antrum [TA], the orifice leading from the epitympanic recess to the mastoid antrum. SYN a. ad antrum mastoideum [TA], a. ad antrum [TA], aperture of mastoid antrum.

a. or'bitae [TA], SYN orbital *opening.*

a. pel'vis, SYN pelvic *inlet.*

ad·just·ment (ă-jŭst'ment). **1.** In dentistry, any modification made upon a fixed or removable prosthesis during or after its insertion to perfect its adaptation and function. **2.** SYN adaptation (6). **3.** A summarizing procedure for a statistical measure in which the effects of differences in composition of the populations being compared have been minimized by statistical methods.

occlusal a., modification of the occluding and incising surfaces of teeth to develop harmonious relationships between these surfaces.

ad·ju·vant (ad'joo-vănt). **1.** A substance added to a drug product formulation that affects the action of the active ingredient in a predictable way. **2.** In immunology, a vehicle used to enhance antigenicity; e.g., a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water-in-oil emulsion in which antigen solution is emulsified in mineral oil (Freund incomplete a.), sometimes with the inclusion of killed mycobacteria (Freund's complete a.) to further enhance antigenicity (inhibits degradation of antigen and/or causes influx of macrophages). **3.** Additional therapy given to enhance or extend primary therapy's effect, as in chemotherapy's addition to a surgical regimen. **4.** A treatment added to a curative treatment to prevent recurrence of clinical cancer from microscopic residual disease. [L. *ad-juvo,* pres. p. *-juvans,* to give aid to]

Freund a., SEE adjuvant.

Freund complete a., water-in-oil emulsion of antigen, to which killed mycobacteria or tuberculosis bacteria are added.

Freund incomplete a., water-in-oil emulsion of antigen, without mycobacteria.

ADL. Abbreviation for activities of daily living. SEE activities of daily living *scale.*

Adler, Alfred, Austrian psychiatrist, 1870–1937. SEE adlerian *psychology;* adlerian *psychoanalysis.*

Adler, Oscar, German physician, 1879–1932. SEE A. *test.*

ad·le·ri·an (ad-ler'ē-an). Relating to or described by Alfred Adler.

ad lib Abbreviation for L. *ad libitum,* freely, as desired.

adm. SEE admov.

ad·me·di·al, ad·me·di
the median plane.

ad·mi·nic·u·lum, pl.
That which gives supp
manus, hand]

a. lin'eae al'bae, a tria
taining a few muscula
ligament to the posteric

ad·mit·tance (ad-mit'a

admov. Abbreviation fc

ad·ner·val (ad-ner'văl).

ad·neu·ral (ad-noor'ăl).
of a nerve; said of an
tissue toward the point

ad·nexa, sing. **ad·nex**
structures, under *struct*
parts]

a. o'culi, SYN accessory

a. u'teri, SYN uterine *ap*

ad·nex·al (ad-nek'săl).

ad·nex·ec·to·my (ad-ne
2. In gynecology, exci
unilateral and excision c
bilateral.

ad·nex·i·tis (ad-neks-ī'ti
annexa, adnexa, + *-itis,*

ad·nex·o·pexy (ad-neks'
the fallopian tube and
plished without suspensi
pēxis, fixation]

Ado Symbol for adenosin

ad·o·les·cence (ad-ō-les'
puberty and ending with
[L. *adolescentia*]

ad·o·les·cent (ad-ō-les'er
individual in that stage o

AdoMet Abbreviation fo

adon·is (a-don'is). Medic
(family Ranunculaceae),
in the treatment of conge
din and related cardioton
Adōnis, mythical figure,

adon·i·tol (ă-don'i-tol). s

ADP Abbreviation for ade

ADPase. SYN apyrase.

adreno-. SEE adreno-.

ad·re·nal (ă-drē'năl). **1.**
suprarenal (adrenal) glan
tissue or product thereof.
kidney]

accessory a., an island
adrenal gland, usually fou
or genital organs. SYN adr

Marchand a.'s, small co
broad ligament of the uter

ad·re·nal·ec·to·my (ă-drē
adrenal glands. [adrenal +

adren·a·line (ă-dren'ă-lin)

a. oxidase, SYN *amine* oxi

ad·re·nal·ism. SYN hyper

adre·nal·i·tis (ă-drē-năl-ī'ti

adren·a·lone (ă-dren'ă-lōn
manufacturing processes;
mology.

adre·na·lop·a·thy (ă-drē-n
of the adrenal glands. SYN
suffering]

ad·ren·ar·che (ad'ren-ar-ki
during puberty induced by

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	Thomas Dag Horn et al.	Group Art Unit: 1642
Serial No.:	10/081,185	Examiner: Gary B. Nickol
Filed:	February 25, 2002	Docket No.: 110.004US2
Title:	IMMUNOTHERAPY OF EPITHELIAL TUMORS USING INTRALESIONAL INJECTION OF ANTIGENS THAT INDUCE A DELAYED TYPE HYPERSENSITIVITY REACTION	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Thomas Dag Horn, declare and say as follows:

1. I am a co-inventor of the subject matter claimed in the above-identified U.S. Patent Application Serial No. 10/081,185, filed February 25, 2002, and of its parent U.S. Patent Application Serial No. 09/344,357, filed June 25, 1999, now U.S. Patent No. 6,350,451, of which U.S. Patent Application Serial No. 10/081,185 is a divisional patent application.

2. I have reviewed the Office Action mailed May 31, 2005 in relation to the above-identified patent application and have reviewed Clements (U.S. Patent No. 6,033,673) cited by the Examiner in the Office Action. I make this declaration in support of the patentability of the claims of U.S. Patent Application Serial No. 10/081,185.

3. I am a Professor of Dermatology and Pathology and the Chairman of the Department of Dermatology in the University of Arkansas for Medical Sciences, Little Rock, AR. I am board certified in dermatology and dermatopathology. I received an M.D. degree from the University of Virginia in 1982, did an internship in pediatrics at the Uniersity of Virginia from 192-1984, a residency in dermatology at the University of Maryland from 1984-1987, and a fellowship in dermatopathology at Johns Hopkins University School of Medicine from 1987-1989. I am an author of several textbooks in

dermatology and dermatopathology and author of over 50 refereed scientific publications in those fields.

4. In my opinion, the E. coli mutant enterotoxin LT(R192G/L211A) is not necessarily antigenic in the compositions disclosed in Clements, and if antigenic, may not induce or be capable of inducing a cutaneous delayed type hypersensitivity response in a human or other mammal.

5. Immune responses fall into two broad categories: humoral, involving the production of soluble antibodies, and cell-mediated, involving proliferation of T cells that bind antigen on the cell surface. Humoral and cell-mediated responses can be further divided into different types of immune response. For instance, an immune response that is both humoral and cell-mediated is a type I hypersensitivity response, which is an immediate hypersensitivity response to an antigen based on IgE antibody and T_H2 cells. Most allergies involve type I responses. A different type hypersensitivity reaction that is a purely cell-mediated response is a type IV hypersensitivity response, also known as delayed-type hypersensitivity.

6. Different antigens and different modes of presentation of the same potentially antigenic substance produce different types of immune response – or no immune response. For instance, ragweed tends to produce a type I hypersensitivity response in persons who develop allergies to it. Poison ivy and tuberculin produce type IV hypersensitivity responses. But many foreign substances presented in certain compositions produce no immune response. The purpose of adjuvants such as Freund's complete adjuvant, is to enhance immune response to other potentially antigenic substances in the composition. Often an antigen can elicit a large immune response when presented with an adjuvant, but no detectable immune response when presented without an adjuvant. Thus, foreign substances that are potentially antigenic do not behave as antigens - that is, they do not induce an immune response - in certain formulations and with certain modes of presentation.

7. Furthermore, when antigens do induce an immune response, the immune response can be in many forms, most of which are not a delayed-type hypersensitivity response. This is evidenced by numerous published papers. To take one example, Lichtenwalner et al., 2004, *Infection and Immunity*, 72:1159-1161, discloses testing

several antigens from Chlamydia for induction of a delayed-type hypersensitivity (DTH) response. Of the antigens, tested, only heat shock protein 60 induced a DTH response, while killed whole organisms, outer membrane protein, and heat shock protein 10 did not. To take another example, Shibata et al., 2001, *Infection and Immunity* 69:6123-6130, reports that immunization of mice with MPD-59 mycobacterial protein without chitin induces certain immune response including IgE production and Th2 cells producing IL-4, IL-5, and IL-10, but does not induce Th1 cells or a delayed-type hypersensitivity response (abstract). To take a third example, Neiwenhuis states, "A major question still remains as to the factors involved in determining whether cellular or humoral immunity will develop in response to a certain antigen." (Nieuwenhuis, P., pp. 3-32, at page 27, in Marsh, J.A. et al. eds., *The Physiology of Immunity*, CRC Press, Boca Raton, FL, 1996.) As that statement implies, different antigens, or antigens presented in different compositions, can lead to an immune response that is exclusively humoral or exclusively cellular, or a combination of both.

8. Accordingly, there is a very strong possibility that in the compositions disclosed in Clements (U.S. Patent No. 6,033,673) the mutant enterotoxin does not induce an immune response to itself at all - that is, is not an antigen. If it does induce an immune response to itself, it is quite likely that the response is a response other than a delayed-type hypersensitivity response. Without testing particular compositions, there is really no way to know what type of immune response if any would be induced.

6. All statements made herein of my own knowledge are true, and all statements made on information and belief are believed to be true. Furthermore, these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and with knowledge that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 8/18/5 By: Thomas Dag Horn

Thomas Dag Horn

52 CCPA

Application of Harry TANCZYN.

Patent Appeal No. 7380.

United States Court of Customs and Patent Appeals.

July 8, 1965.

Proceeding in the matter of an application for a patent. The Board of Appeals of the United States Patent Office, Serial No. 854,226, affirmed examiner's rejection of all claims of application, and the applicant appealed. The Court of Customs and Patent Appeals, Almond, J., held that claims of application for patent for stainless steel containing both nitrogen and molybdenum were erroneously rejected on ground of obviousness in view of prior art.

Reversed.

1. Patents ⇐18

Where rejection of patent application is based on obviousness, purpose of affidavit of prior invention relied on to overcome cited patent or publication is to establish that claimed invention was made by applicant before effective date of reference relied on to show that invention was obvious. 35 U.S.C.A. § 103; Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

2. Patents ⇐73

References may not be overcome by affidavits showing that applicant had invented, prior to reference date, a part, some parts, or even combination of parts used to create embodiment of his claimed invention, where part or parts are not within scope of claims being sought. Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

3. Patents ⇐91(3)

Affidavit relied upon by patent applicant to antedate reference need not show entire claimed invention in all cases except genus-species situations. Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

4. Patents ⇐91(3)

It is not sufficient to show in affidavit relied upon by patent applicant to antedate reference that invention wholly outside of that being claimed was made prior to reference date. Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

5. Patents ⇐91(4)

Affidavit, which was relied upon by applicant for patent for invention of stainless steel containing both molybdenum and nitrogen to antedate reference, which established that prior to filing date of reference, applicant made nitrogen containing stainless steel having same composition as that disclosed by reference, but which did not disclose completion of claimed invention or a part thereof prior to date when reference was available was not effective to overcome reference disclosing stainless steel differing from applicant's steel only in that it did not contain molybdenum. 35 U.S.C.A. § 103; Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

6. Patents ⇐18

Claims of application for patent for stainless steel containing both nitrogen and molybdenum were erroneously rejected on ground of obviousness in view of prior art. 35 U.S.C.A. § 103.

John Howard Joynt, Washington, D. C., for appellant.

Clarence W. Moore, Washington, D. C. (Fred W. Sherling, Washington, D. C., of counsel), for the Commissioner of Patents.

Before WORLEY, Chief Judge, and RICH, MARTIN, SMITH and ALMOND, Judges.

ALMOND, Judge.

Harry Tanczyn appeals from a decision of the Board of Appeals affirming the rejection of all claims in appellant's application.[1] Appellant's invention relates to a stainless s
produced below

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stainless steel
itation-harde
treatment fro
stenitic conc
strength in c
ductility, said
tially of 9.00%
2.50% to 8.
2.50% alumi
lybdenum, wi
mium and mol
to 21%, .10%
bon .12% r
8.00% maxin
inversely pro
to the nickel
maximum, ph
mum, sulphur
remainder sul

Appellant's st
art in that it co
molybdenum. T
on by the exami

Goller 2,50
Walton 2,89

Goller disclose
the same compo
cept that it cont
ton discloses a s
fers from appel
fact that it does
The examiner's
molybdenum an
steel would be o
and Walton.

Appellant filed
131 in an attemp
reference. The
davit was not e
reference becaus
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part thereof *
if the board err
decision must be
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Thus, we shall
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1. Serial No. 854,226, filed November 20, 1959, for "Stainless Steel and Method."

to a stainless steel of which claim 1 reproduced below is illustrative.

> A chromium-nickel-aluminum stainless steel susceptible to precipitation-hardening by double heat-treatment from a soft, workable austenitic condition to give great strength in combination with good ductility, said steel consisting essentially of 9.00% to 20.00% chromium, 2.50% to 8.00% nickel, .70% to 2.50% aluminum, 1% to 5% molybdenum, with the sum of the chromium and molybdenum contents 14% to 21%, .10% to .40% nitrogen, carbon .12% maximum, manganese 8.00% maximum, with manganese inversely proportioned with respect to the nickel content, silicon 2.00% maximum, phosphorus .050% maximum, sulphur .050% maximum, and remainder substantially all iron.

Appellant's steel differs from the prior art in that it contains both nitrogen and molybdenum. The references relied upon by the examiner are:

Goller	2,505,763	May 2, 1950
Walton	2,892,702	June 30, 1959

Goller discloses a stainless steel having the same composition as appellant's except that it contains no nitrogen. Walton discloses a stainless steel which differs from appellant's steel only in the fact that it does not contain molybdenum. The examiner's rejection was that a molybdenum and nitrogen containing steel would be obvious in view of Goller and Walton.

Appellant filed an affidavit under Rule 131 in an attempt to antedate the Walton reference. The board held that the affidavit was not effective to antedate the reference because it did not show "completion of the claimed invention or any part thereof * * *." It is clear that if the board erred in this holding the decision must be reversed since a rejection on Goller alone cannot be sustained. Thus, we shall first consider the adequacy of the affidavit.

The Rule 131 Affidavit

The affidavit establishes that prior to January 4, 1955, the filing date of the Walton patent, appellant made a nitrogen containing stainless steel having the same composition as that disclosed by Walton. The affidavit does not disclose completion of the *claimed* invention prior to the date when both Goller and Walton were available.

The examiner, citing Ex parte Blair, 129 USPQ 424 (P.O.B.A.1957), held that the affidavit was directed to an invention which differs in kind from the claimed invention and therefore that no weight could be attached to the affidavit. It is the appellant's position that Blair "merely harked back to the views of the Board, as reversed by this court" in In re Stempel, 241 F.2d 755, 44 CCPA 820.

When a rejection under 35 U.S.C. § 102(a) is involved, we have held that "under the law all the applicant can be required to show is priority with respect to so much of the claimed invention as the reference happens to show." In re Stempel, 241 F.2d at 759, 44 CCPA at 826. In Stempel the claims under appeal were drawn to *chemical compounds*. Both genus and species claims were present. One of the claimed species was disclosed in a U. S. patent. The reference was antedated by affidavits under Rule 131 which established that appellant had made the species disclosed in the reference before the effective date of the reference. The Board of Appeals held that establishing priority to a common species was not sufficient to obtain allowance of a generic claim. This court reversed the board, holding that a Rule 131 affidavit need not show the invention "*defined in the claim the applicant is asking for* * * *." 214 F.2d at 759, 44 CCPA at 825.

In Stempel the applicant successfully overcame the reference as to both generic and species claims by showing priority only as to the species shown by the reference. It was, however, a species *of the claimed generic invention*. The species of the reference and affidavit was sufficient to anticipate the generic claims of

the application. Thus, in addition to establishing priority as to the species of the reference, the Stempel affidavit also disclosed completion of the invention, although admittedly not in the broad generic form. The reduction to practice shown was of a species of the invention within the generic claims.

[1] A different situation may prevail when the rejection is based upon 35 U.S.C. § 103. In such a case the purpose of an affidavit is to establish that the claimed invention was made by the applicant before the effective date of a reference relied upon to show that the invention was obvious. By so doing, the applicant may prove, by eliminating a reference, that at the time the invention was made "the subject matter as a whole would [not] have been obvious * * *."

In Ex parte Blair, supra, a rejection on a combination of two references under 35 U.S.C. § 103 was involved. By a Rule 131 affidavit, the applicant antedated one of the references. Although the affidavit did not show completion of the claimed invention, it did show completion of that "part" of the claimed invention which the reference showed. The appellant argued that such a showing was sufficient under Stempel. The board held the affidavit ineffective, stating:

> It seems to us that the mere fact that an inventor may have invented the combination, A, B, and C, for example, before a patentee does not in itself prove that he has also invented the combination, A, B, C, and D at the same time (D representing the extra pair or pairs of fingers over the ten pairs shown by Schon).
>
> In our opinion, the term "invention" in Rule 131 is used in the same sense as in the Patent Statute to mean that which is *claimed* as new and patentably useful, 35 U.S.C. Sections 101, 102, 103, and 112. Section 112 requires that:
>
> "The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention."

and *it is this claimed "invention" which we think was intended in Rule 131.* It does not appear to us logical that one should be permitted to dissect the invention, in this instance the claimed *combination, into several parts* and then say because he has invented *one* such *part* prior to a *reference disclosing that part* that he has also invented the entire combination prior to that reference, even though other references combined therewith to meet "the invention" bear an earlier date. [Emphasis added.]

We are in general agreement with the reasoning of the board although not necessarily with its precise language. It must be remembered that in Stempel the dispute was whether it was completion of the *claimed* invention—specifically, the genus of the generic claims—that had to be established prior to the date of the reference, and we held that it did not where the reference did not disclose that invention but only a species within it.

The mere fact that an applicant has previously produced that which is disclosed by a reference, however, may have no bearing on the problem of whether he made his invention or a patentable portion of it before the date of a reference.

In retrospect and in the light of the facts now before us, it seems necessary to comment on and to restrict somewhat certain broad language in Stempel, particularly the two following statements:

> We are convinced that under the law all the applicant can be required to show is priority with respect to so much of the claimed invention as the reference happens to show. When he has done that he has disposed of the reference.
>
> * * * * * *
>
> In the case of a reference, it is fundamental that it is valid only for

> what it discloses and if the applicant establishes priority with respect to that disclosure, and there is no statutory bar, it is of no effect at all.

Those statements were entirely valid, we still think, *as applied to the facts in Stempel* where the reference showed a species of the generic invention being claimed in the appealed claims. As frequently happens, however, the statements are broad enough to encompass the very different situation we now have before us and which was present in Blair. There is a difficulty in discussing it because of the ambiguity of the word "part" in speaking of "part of an invention." It can mean such a "part" as the species which Stempel invented, which was part of his claimed genus. It can mean an element or subcombination of elements forming part of a larger combination of elements making up a machine such as the bowling pin spotting device of Blair. Or it can mean an alloying ingredient or an alloy containing all but one of the essential ingredients, as here. In one sense, a stainless steel alloy containing nitrogen but no molybdenum is a part, even a large part, of appellants' alloy; in a more accurate sense, however, it is not a "part" *of his invention* at all because his invention does not exist at all until there is an alloy containing *both* nitrogen and molybdenum.

[2] We never intended by the language used in Stempel to authorize the overcoming of references by affidavits showing that the applicant had invented, prior to the reference date, a part, some parts, or even a combination of parts, used to create an embodiment of his claimed invention, where the part or parts are not within the scope of the claims being sought, as the species of Stempel shown by the reference was within his generic claims.

In our view, appellant was never in possession of any "part" of his claimed invention until he was in possession of the stainless steel alloy combination containing both nitrogen and molybdenum. Using the term "part" in that same sense, neither reference shows "part" of his invention.

For these reasons we are in general agreement with the board's disposition of the issue and with its statement that:

> The instant case does not involve a genus-species relationship. It is abundantly clear from appellant's specification and claims that his invention is in the combined addition of molybdenum and nitrogen to a hardenable chromium-nickel aluminum stainless steel of low carbon content which is not disclosed in the affidavit.

[3] While this succinct statement of the board's position appears correct, it might be read to imply that not only must the affidavit show everything the reference shows but also that the *entire* claimed invention must be shown in *all cases* except the genus-species situation. We will not now sanction such a broad rule. It is contrary to Stempel. The primary consideration is whether, in addition to showing what the reference shows, the affidavit also establishes possession of either the *whole* invention claimed or something falling *within* the claim, in the sense that the claim as a whole reads on it.

[4, 5] It is not sufficient to show in a Rule 131 affidavit that an invention wholly outside of that being claimed was made prior to the reference date. Such fact is irrelevant. We therefore hold the Rule 131 affidavit herein to be ineffective to overcome the Walton reference [2] and turn to the question of obviousness, taking that reference into consideration.

Obviousness

We must now consider whether appellant's claimed invention is obvious within the meaning of 35 U.S.C. § 103.

[2] See Pearlman, "Plea for a Realistic Approach to Rule 131 Practice," 43 JPOS 844 (1961), which we have considered and also the Drake commentary thereon at 44 JPOS 212, to which Pearlman replied at 44 JPOS 424.

Both references disclose chromium-nickel stainless steel. Goller discloses that the stainless steel may contain

> * * * anywhere from about 0.02% to 0.12% carbon, aluminum from about 0.50% to 2.50%, from incidental amounts up to about 8.0% manganese, from incidental amounts up to approximately 2.0% silicon, with or without molybdenum ranging up to about 3.0% illustratively to enhance corrosion resistance of the steel, and the remainder substantially all iron. * * *

The Walton stainless steel contains from 0.12% to 0.18% nitrogen to "prevent hardening following solution annealing and render the same uniformly responsive to transforming and precipitation hardening treatments."

The board held that appellant's claimed invention was obvious, stating:

> Goller discloses a hardenable chromium-nickel-aluminum stainless steel in which a portion of the chromium content may be replaced with molybdenum to enhance its corrosion resistance. In our view it would be obvious to a person of ordinary skill in the art to replace a portion of the chromium in the similar stainless steel alloy of Walton et al. for the purpose described by Goller. Conversely, Walton et al. disclose that the addition of nitrogen to a hardenable chromium-nickel aluminum stainless steel will prevent undesirable hardening following solution annealing and it appears to us that the addition of nitrogen to the similar alloy of Goller for the same purpose would also be obvious.

Appellant points out that his invention is not a chromium-nickel stainless steel having outstanding corrosion resistance but rather is a stainless steel which possesses a combination of strength and ductility. The disclosed properties of appellant's steel and the reference steels are as follows:

Property	Appellant	Walton	Goller
Tensile strength	200,000 psi	165,000 psi	214,000 psi
Elongation	16%	8%	6%
Reduction in Area	54%	—	21%

Appellant argues that a steel having these properties is not obvious from the cited references. The board did not answer this contention directly but rather found fault with the comparison as follows:

> We are not impressed with this comparison because there is no direct basis for comparing appellant's steel with those of the references. The heat treatment employed by Goller is different from the heat treatment used in hardening the steels of appellant's Table II. Walton et al. do not disclose the specific heat treatment employed in obtaining the values given. * * *

The appellant rebuts this criticism as follows:

> There is nothing in either of the reference citations, or indeed in appellant's disclosure, pointing to any criticality in the heat treatments employed; they all use the combination of solution treatment, preliminary hardening treatment, and final precipitation-hardening treatment wherein a *range* of treating temperatures and a range of time of treatment are acceptable. In Goller the solution annealing is conducted at 1800° to 2000° F., the preliminary hardening at 1200° to 1600° F., preferably 1400° F., and the final hardening at 750° to 1000° F. * * * in Walton et al. these three treatments are respectively conducted at 1800° to 2000° F., 1300° to 1500° F.

and 700° to 1100° F. * * *; and in appellant's disclosure the three are conducted at 1800° to 2100° F., 1400° F., and 750° to 1100° F., respectively * * *.

We agree with appellant that the board presented no convincing reason why the alloys cannot be compared. Although differences in heat treatment do exist, there is nothing before us to suggest that these slight differences would affect both strength and ductility.

The solicitor treats the improved properties in a somewhat different manner, stating:

Certainly, the references show that it is known that steels containing either molybdenum or nitrogen have good elongation, and appellant has not shown that the improved elongation of his steel is anything other than the expected additive effects of molybdenum and nitrogen. * * *

We are at a loss to know where the references indicate that the additive effect of molybdenum and nitrogen is improved elongation. We are of the view that the combination of strength and ductility would be unexpected from the cited prior art.

The solicitor further argues that even the elongation is unexpected, the claimed alloy is obvious because it is obvious to make an alloy containing both nitrogen and molybdenum for their desired properties. In this regard we note in reading the references cited by the Patent Office that the formulation of a suitable alloy may not be done merely by adding one element characterized as a strength additive and another as an elongation additive. For example, Goller notes that aluminum has been recognized as a deoxidizing material. However, aluminum was considered to impair hardenability but in the Goller composition aluminum aided hardening. Two columns in the Goller patent describe various additives that may be included and the delicate balance that must be maintained between these additives. Walton teaches that the reuse of various alloys to aging treatments varies widely for reasons unknown. In view of this reported difficulty in balancing the properties and compositions of alloys, it appears that the solicitor's approach is unrealistic.

[6] In the absence of either a suggestion in the cited art that nitrogen and molybdenum should be combined in one alloy or of an alloy having properties similar to appellant's, we hold that the claimed composition is unobvious within the meaning of 35 U.S.C. § 103.

The decision of the board is reversed.

Reversed.

52 CCPA

Frederic O. HESS, Appellant,

v.

Charles C. BLAND, Appellee.

Patent Appeal No. 7414.

United States Court of Customs and Patent Appeals.

July 15, 1965.

Patent interference proceeding. The Board of Interference Examiners of the United States Patent Office, Interference No. 91,619, awarded priority of invention to senior party, and junior party appealed. The Court of Customs and Patent Appeals, Martin, J., held that evidence did not establish actual reduction to practice by junior party of invention relating to process for making glass beads or spheres for reflecting tape and paint.

Affirmed.

1. Patents ⇐91(3)

In interference proceeding between copending applications, junior party had burden of proving his case by preponderance of the evidence.

the conclusion that the *degree* of analgesic potency exhibited by one compound of the genus establishes the degree of potency of the genus or any other of its members. Accordingly, appellant's arguments for patentability based upon the degree of analgesic activity are considered pertinent only to claim 4, which defines the only compound for which datum has been provided.

It should be noted that no data are available for piperidine esters which differ *only* in the arrangement of the 4-ester group. Thus, generalizations based upon the data set forth in the above table are at best inconclusive. The data tend to indicate that "reverse" esters are more potent analgesics than "normal" esters when the standard is meperidine hydrochloride, but the opposite trend is indicated when the standard is the 1-benzyl compound. One thing is clear, and that is that the prior art suggests that the compound of claim 4 would be an analgesic. While the compound of claim 4 has a significantly greater potency than the tested Carabateas claimed "reverse" esters, the above table reveals that the former compound differs from the latter compounds by at least one methylene group, in one instance the difference being in the "A" substituent. Conceivably, these methylene groups have a significant influence on analgesic potency.

[2] Thus, it cannot be determined with any degree of certainty to what the increased potency is attributable. We are unable to find "clear and convincing evidence," as required by this court in In re Lohr, 317 F.2d 388, 50 CCPA 1274, that appellant's compounds possess unexpected activity compared to the *closest* Carabateas reference compound. Because of appellant's lack of proof, the decision of the board is affirmed.

Affirmed.

SMITH, J., concurs in the result.

53 CCPA

Application of Fritz HOSTETTLER and Eugene F. Cox.

Patent Appeal No. 7564.

United States Court of Customs and Patent Appeals.

Feb. 17, 1966.

Rehearing Denied May 5, 1966.

Proceeding on patent application No. 24,650 relating to a tin-containing catalyst for isocyanate reactions. From a decision of the Board of Appeals affirming examiner's rejection of claim, the applicants appealed. The United States Court of Customs and Patent Appeals, Martin, J., held that evidence was sufficient to establish that one of ordinary skill in the art would be satisfied from facts shown in affidavit in patent application that applicants had completed invention as defined in claims, and applicants should not have been required to submit facts showing that they reduced to practice that which was obvious in addition to those facts offered as showing a completion of the invention, for the purposes of antedating a reference.

Reversed.

1. Patents ⇐98

Rule requiring applicant for patent to make oath to facts showing a completion of invention does not mean affiant must show a reduction to practice of every embodiment of the invention, nor is that requirement coextensive with amount of disclosure necessary to support a claim under specification portion of statute. Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.; 35 U.S.C.A. § 112.

2. Patents ⇐91(4)

Evidence was sufficient to establish that one of ordinary skill in the art would be satisfied from facts shown in affidavit in patent application relating to a tin-containing catalyst for isocyanate reactions that applicants had completed invention as defined in claims, and applicants should not have been required to

submit facts showing that they reduced to practice that which was obvious in addition to those facts offered as showing a completion of the invention, for the purposes of antedating a reference. Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.; 35 U.S.C.A. §§ 102(a), 112.

Charles J. Metz, Francis M. Fazio, New York City, Paul A. Rose, Washington, D. C., Louis C. Smith, New York City, for appellants.

Joseph Schimmel, Washington, D. C. (George C. Roeming, Washington, D. C., of counsel), for the Commissioner of Patents.

Before RICH, Acting Chief Judge, and MARTIN, SMITH and ALMOND, Judges, and WILLIAM H. KIRKPATRICK.*

MARTIN, Judge.

The issue for determination in this appeal [1] from the Board of Appeals is the sufficiency of an affidavit under Rule 131 to remove publications which are conceded to be prior art references under 35 U.S.C. § 102(a).

The invention is evident from the sole claim in the case on appeal here:

> 4. A process for producing a urethane which comprises reacting (a) a compound having at least one isocyanato group with (b) a compound having at least one alcoholic hydroxyl group, in the presence of a catalytic amount of stannous octoate, wherein the sole reactive groups present in both said compounds are isocyanato and aliphatic alcoholic hydroxyl groups, respectively.

It should be noted that the process claim, in calling for each reactant having "at least one" of the pertinent reactive groups, encompasses the use of alcohols and isocyanates of any "functionality" as reactants to produce compounds having a *single* NHCOO linkage, as well as those having a plurality of such linkages such as *poly*urethane resins and foams.[2] The reaction of the claimed process can be depicted in simplified form by the equation:

$$R^1 - NCO + HO - R^2 \xrightarrow[\text{Stannous Octoate}]{} R^1 - NH - \overset{\overset{O}{\|}}{C} - O - R^2$$

Isocyanato Group Containing Reactant	Hydroxyl Group Containing Reactant	Urethane Group Containing Product

R^1 and R^2 represent various organic moieties.

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* United States Senior District Judge for the Eastern District of Pennsylvania, designated to participate in place of Chief Judge WORLEY, pursuant to provisions of Section 294(d), Title 28, United States Code.

1. This appeal PA 7564, is taken on application serial No. 24,650, filed April 26, 1960 for "Tin-Containing Catalyst for Isocyanate Reactions."

2. Functionality in this context thus refers to the reactive or functional groups of the reactants. "Any functionality" means having one or any number of functional groups per reactant molecule.

The references relied on for the § 102 (a) rejection are:

Technical Information Bulletin, No. 28–F9, July 20, 1959 Mobay Chemical Co., Pittsburgh, Penna.

Modern Plastics, Feb. 1960; page 53.

There is no issue as to what those references show since the solicitor agrees that appellants adequately summarize them in their brief thus:

> The references * * * disclose the use of stannous octoate as a catalyst in the production of urethane foams which involves the reaction of polyfunctional alcohols with polyfunctional isocyanates to form polyurethanes. Thus, the references disclose the subject claimed invention employing polyfunctional reactants.

The Rule 131 affidavit alleges a completion of the invention prior to May 26, 1958,[3] which is prior to the effective date of the earliest reference. In the affidavit, reference is made to accompanying notebook pages on which was recorded an experiment using stannous octoate to catalyze the reaction of a *mono*functional alcohol (methanol) with a *mono*functional isocyanate (phenyl isocyanate) to produce a urethane containing only one NHCOO—group (methyl N-phenylcarbamate). The subject of the research is stated on the notebook pages to be "Catalytic Studies of the Reaction of Isocyanates with Active Hydrogen Compounds * * *," and the purpose of the particular experiment was "to determine the velocity coefficient for the [above noted] reaction * * using * * * Stannous Octoate * * as catalyst." The affidavit admittedly does not show facts establishing reduction to practice of a process involving use of stannous octoate to produce a polyurethane resin or foam.

The examiner was not satisfied by the affidavit, stating both in the Final Rejection and in the Answer:

> * * * Applicants' attention is directed to the premise of M.P.E.P. section 715.03 which states that the rejection is valid unless applicant overcomes the exact species of the reference by his affidavit, or else the affidavit shows an adequate generic disclosure. Applicants have failed to do this since their affidavit covers only one species methanol which is in fact generically different from the reference's species. As a matter of fact, the references all concern the formation of polymers, whereas the affidavit shows the reaction of methanol, a monofunctional compound with monoisocyanate, said reaction being unable to form a polymer. Thus the Stempel case [In re Stempel, 241 F.2d 755, 44 CCPA 820, 113 USPQ 77] is not applicable.

The board in affirming stated:

> In essence, the affidavit proves a reduction to practice of the simple monomeric reaction whereby methyl N-phenylcarbamate is produced from phenyl isocyanate and methanol, using stannous octoate as a catalyst. This may have been a useful preliminary or screening experiment raising hopes that the catalyst would be correspondingly useful in the preparation of urethane polymers, but it cannot be regarded as a reduction to practice of that principal aspect of the claimed process which involves the production of polyurethanes, particularly polyurethane foams. The simple monomer experiment is not deemed to be representative of the problems encountered in the preparation of polyurethane foams * * *, and certainly does not demonstrate the

3. That date coincides with the filing date of a patent to Ikeda, No. 3,010,923 issued November 28, 1961, which was cited of interest in the prosecution but not relied on in the appeal.

beneficial results mentioned in * * appellants' specification.

Appellants may have carried out the simple reaction of their affidavit prior to the cited publications, but they did not complete the invention of the scope here claimed or the foamed polyurethane aspect taught in the references prior to the date of the references. Chronologically, appellants reacted phenyl isocyanate and methanol in the presence of stannous octoate and obtained methyl N-phenylcarbamate. Thereafter, the cited references were published disclosing the preparation of polyurethane foams using stannous octoate as the catalyst. Finally, the instant application was filed disclosing and claiming all aspects of urethane production using the specified catalyst. The record furnishes no basis for any different conclusion as to the chronological course of events and, clearly, it cannot be held that appellants were first as to all aspects of urethane production using stannous octoate as the catalyst.

We are not impressed by appellants' arguments that the procedure of their affidavit is representative of urethane production in general. The simple monomer reaction may be indicative of possible future utility of stannous octoate in the preparation of polyurethane foams, but the reaction is not so nearly identical that the completion of the simple monomer reaction can be regarded as constituting a completion of the considerably more complex polymer reaction.

The solicitor urges that the above position is particularly correct in view of the generally known unpredictability of catalytic activity, citing: Corona Cord Tire Co. v. Dovan Chemical Corp., 276 U.S. 358, 48 S.Ct. 380, 72 L.Ed. 610 (1928); In re Doumani, 281 F.2d 215, 47 CCPA 1120; Ex parte Meguerian, 124 USPQ 456 (Pat.Off.Bd.App.1960); Ex parte Fugate, 99 USPQ 54 (Pat.Off. Bd.App.1953).

Thus, the issue, more specifically stated, is whether the factual showings in the Rule 131 affidavit relating the catalysis of a process to produce a compound having a single urethane group is sufficient to antedate a reference under § 102(a) which shows the claimed process as producing polyurethane resins or foams.[4]

[1] We think the board erred in its view of what is the invention, and thereby demanded appellants show more in the affidavit than is necessary under Rule 131. Rule 131 requires applicant to make oath to facts showing a completion "of the invention." That requirement does not mean affiant must show a reduction to practice of every embodiment of the invention. Nor is that requirement coextensive with the amount of disclosure necessary to support a claim under 35 U.S.C. § 112.

The invention here is the use of a catalyst in a process involving an old reaction. As appellants state:

> * * * But has not the Board lost sight of that which appellants seek to claim, i. e., the use of a new catalyst for an old reaction? The Board emphasizes the functionality of the reactants, and thus whether or not the reactants are polymer-forming. But the Board has ignored the fact that, regardless of

4. There is clearly no issue as to whether the specification is adequate to support the claim or that the claim is unduly broad, since the board reversed the examiner on that issue, stating:

> In our view, the nature of appellants' disclosure and the state of the prior art in the present case does not require that the known reactants be defined with greater particularity. We will not sustain the rejection of the appealed claim under 35 U.S.C. 112.

See In re Fong, 288 F.2d 932, 48 CCPA 897, 903.

> functionality, alcohol plus isocyanate produces urethane, and it is the use of stannous octoate to catalyze this urethane-forming reaction that appellants seek to claim. The invention is predicated upon catalytic activity alone. The Rule 131 Affidavit * * * shows such catalytic activity thereby overcoming references whose sole *pertinent* disclosure is the same catalytic activity.

The apparent requirement of the Patent Office that appellants show production of other *products*, polyurethane resins or foams, is considered improper in view of the nature of the invention. See In re Fong, 288 F.2d 932, 48 CCPA 897, 902. As evidence that alcohols of any functionality can be reacted with isocyanates of any functionality, appellants rely on a patent to Rothrock, No. 2,374,136 issued April 17, 1945,[5] which states in pertinent part:

> * * * When the active hydrogen-containing substances [sic] is monomeric and contains only one active hydrogen-containing group, the product is in general monomeric. On the other hand, if both of the reactants are bifunctional, the product is polymeric; and, if one of the reactants is polymeric, the product is a modified polymer of higher molecular weight. * * *

The examiner's response, referred to by the board, was:

> * * * Applicants cite Example I [in Rothrock] as a "model reaction" similar to applicants', in that the effectiveness of the catalyst is established, without the formation of the polymer, by the reactants. Applicants have apparently closed their eyes to the other specific examples, wherein the results of using or not using the catalysts are disclosed, not with methanol and a diisocyanate, but with a diisocyanate and a bifunctional high polymer forming polyhydroxyl containing compound.

Clearly, while there is a difference shown in that patent between "using or not using the catalysts * * *," as the examiner stated, the other specific examples support the position of appellants: that a catalyst for the monofunctional reactants also catalyzes the reaction between polyfunctional reactants. We think that is the gist of the teaching of Rothrock with regard to catalysis.

[2] It is clear on this record that one of ordinary skill in this art would consider that functionality of the reactants determines whether the products are "monomeric"[6] or polymeric, but not that functionality would matter insofar as the reaction using the catalyst is concerned. The statement as to unpredictability of catalytic activity, while relevant, is so general as to afford little assistance in the determination of the precise issue before us. In fact, the more specific showings in the affidavit and the Rothrock patent indicate that one of ordinary skill in this art would expect that a catalyst for the particular functional groups involved in the reaction would operate relatively independently of the number of those groups on the reactant molecule. Thus we conclude that one of ordinary skill in the art would be satisfied from the facts shown in the affidavit that appellants had completed *the invention* as defined in the claims. See In re Fong, supra. Certainly appellants should not be required to submit facts under Rule 131 showing that they reduced to practice

5. While this patent was cited during the prosecution but not relied on for the rejection, the state of the prior art is not restricted to the documents relied on by the Patent Office. They must, however, be produced before the Patent Office. In re Cofer, 354 F.2d 664, 53 CCPA —.

6. By "monomeric" we mean a product containing only one NHCOO—or urethane group, and do not use the word in its technically more narrow sense as applied to a vinyl group-containing compound in addition polymerization.

that which is obvious in addition to those facts offered as showing a completion of the invention, for the purposes of antedating a reference.

For the foregoing reasons the decision of the board is reversed.

Reversed.

53 CCPA

The SEVEN-UP COMPANY

v.

TROPICANA PRODUCTS, INC.

Patent Appeal No. 7581.

United States Court of Customs and Patent Appeals.

March 3, 1966.

The Trademark Trial and Appeal Board of the United States Patent Office dismissed application No. 41,066 to register a mark. The opponent appealed. The Court of Customs and Patent Appeals, Kirkpatrick, J., held that the mark "SUN-UP" for orange concentrate used in preparation of uncarbonated orange drink was not so confusingly similar to marks "SEVEN-UP" or "7-UP" for carbonated beverage having a lemon-lime flavor as to permit denial of registration of mark "SUN-UP".

Affirmed.

Martin, J., dissented.

1. Trade Regulation ⇐188

The mark "SUN-UP" for orange concentrate used in preparation of uncarbonated orange drink was not so confusingly similar to marks "SEVEN-UP" or "7-UP" for carbonated beverage having a lemon-lime flavor as to permit denial of registration of mark "SUN-UP". Lanham Trade-Mark Act, § 2, 15 U.S.C.A. § 1052.

2. Trade Regulation ⇐182

The meaning of words in trademarks is often an important consideration in determining whether likelihood of confusion exists; where words have well known and understood, widely differing meanings, small difference in spelling or appearance may be sufficient to distinguish them and avoid finding of confusing similarity; on the other hand, with coined words which are meaningless so far as English language is concerned, slight variations in spelling or arrangement of letters are often insufficient to direct buyer's attention to distinction between marks. Lanham Trade-Mark Act, § 2, 15 U.S.C.A. § 1052.

Lewis S. Garner, Beverly W. Pattishall, Helen W. Nies, Chicago, Ill., for appellant.

C. Willard Hayes, Washington, D. C. (Cushman, Darby & Cushman, Washington, D. C., of counsel), for appellee.

Before RICH, Acting Chief Judge, and MARTIN, SMITH, and ALMOND, Judges, and Judge WILLIAM H. KIRKPATRICK.*

KIRKPATRICK, Judge.

[1] This is an appeal from the decision of the Trademark Trial and Appeal Board dismissing an opposition to Tropicana Products, Inc.'s application to register the mark "SUN-UP" for orange concentrate used in the preparation of uncarbonated orange drink. The product is sold mainly to dairies and distributed through dairy route men to householders

* United States Senior District Judge for the Eastern District of Pennsylvania, designated to participate in place of Chief Judge Worley, pursuant to provisions of Section 294(d), Title 28, United States Code.

case was submitted, amounting to no more than a statement of disagreement with the board, having heard oral argument for the Patent Office, and finding no error in the decision below, it is affirmed.

Affirmed.

58 CCPA

Application of Abner B. STRYKER, Jr.

Patent Appeal No. 8420.

United States Court of Customs and Patent Appeals.

Jan. 14, 1971.

Appeal from decision of the Board of Appeals of United States Patent Office, Serial No. 272,449, affirming rejection of both claims in application for improved process for producing polypropylene. The Court of Customs and Patent Appeals, Lane, J., held that where difference between claimed invention and reference disclosure were so small as to render claims obvious over reference, antedating affidavit submitted on claimant's behalf by representative of assignee of application removed reference disclosure as a reference notwithstanding affidavit, while alleging conception and reduction to practice of claimed improved process, including weight percentage limitations, before reference filing date, failed to show corroborating evidence of such weight percentage limitations.

Reversed.

1. Patents ⇐18

Claimed invention of flashing a suspension containing about 50–60 percent by weight polypropylene in liquid propylene to obtain a polymer containing not in excess of about two percent by weight propylene did not produce any unexpected results and it did not render claimed process unobvious over prior process involving separation of polypropylene from propylene by flashing of monomer from polymer in cyclone-type flash zone using mixture containing 35 percent solids by weight.

2. Patents ⇐18

Where difference between claimed invention and reference disclosure were so small as to render claims obvious over reference, antedating affidavit submitted on claimant's behalf by representative of assignee of application removed reference disclosure as a reference notwithstanding affidavit, while alleging conception and reduction to practice of claimed improved process for producing polypropylene, including weight percentage limitations, before reference filing date, failed to show corroborating evidence of such weight percentage limitations. 35 U.S.C.A. § 103.

Fred S. Valles, Ronald J. Carlson, Paramus, N. J., attorneys of record, for appellant.

S. Wm. Cochran, Washington, D. C., for the Commissioner of Patents; Jack E. Armore, Washington, D. C., of counsel.

Before RICH, ALMOND, BALDWIN, and LANE, Judges, and NEWMAN, Judge, United States Customs Court, sitting by designation.

LANE, Judge.

This appeal is from the decision of the Patent Office Board of Appeals, which affirmed the rejection of both claims in appellant's application serial No. 272,449, filed April 11, 1963, for an improved process for producing polypropylene. We reverse.

The invention is defined, and also adequately described for our purposes, by claim 1:

> The process of removing propylene diluent from a suspension consisting essentially of from about 50%–60% by weight polypropylene in liquid propylene obtained directly from a propylene polymerization reactor under the autogenous pressure of the reactor which consists essentially in feeding the said suspension from the reactor to a recovery zone of the cyclone type

maintained at substantially atmospheric pressure, whereby propylene diluent is flashed from the solid particles of polypropylene, leaving on said particles not in excess of about 2% by weight propylene, and recovering the thus treated polypropylene.

The claims were rejected as obvious over Harban,[1] who discloses separation of polypropylene from propylene by flashing of monomer from polymer in a cyclone-type flash zone. The Harban mixture is disclosed as containing 35% solids by weight, and the patent indicates that the separation achieved is nearly perfect, but does not state a specific percentage of separation.

[1] Appellant contends that he discovered that, unexpectedly, the use of a suspension containing 50–60% by weight polymer permitted direct discharge of the suspension into a flashing zone maintained at atmospheric pressure, and resulted in a residual monomer level of 2% or less. We have considered appellant's arguments on this point, but we agree with the Patent Office that appellant's polymer concentration does not produce any unexpected results and does not render the claimed process unobvious over Harban.

We turn now to appellant's alternative contention, i.e., even if the claimed processes are obvious over Harban, Harban is removed as a reference by the antedating affidavit submitted on appellant's behalf by a representative of the assignee of the application.[2] The board considered the affidavit deficient in that, while it alleged conception and reduction to practice of the claimed process, including the weight percentage limitations, before the Harban filing date, there was no corroborating evidence showing those weight percentage limitations. The board stated:

> The *claimed* invention must be shown in the affidavit, i.e., the alleged essence of the invention of flashing a suspension containing about 50%–60% by weight polypropylene in liquid propylene to obtain a polymer containing not in excess of about 2% by weight propylene must at least be demonstrated therein. In re Tanczyn, 52 CCPA 1630; 146 USPQ 298, 347 F.(2d) 830; 821 OG 849 [1965]. (Original emphasis.)

[2] We think the board erred in applying *Tanczyn* to the facts of this case. In *Tanczyn* we limited to a certain extent the language we used in In re Stempel, 241 F.2d 755, 44 CCPA 820 (1957), wherein we had stated that "all the applicant can be required to show is priority with respect to so much of the claimed invention as the reference happens to show." It will be recalled that in *Tanczyn* the appellant sought to remove a reference by an affidavit which showed prior possession by the appellant of subject matter on which the claims did not read but which corresponded to the subject matter disclosed in the reference sought to be removed. We found the affidavit insufficient to remove the reference, but we held the claims to be unobvious over the reference. In other words, the subject matter shown in the reference and the affidavit was so different from the claimed invention that the claims were unobvious and patentable over the reference. In the case before us the differences between the claimed invention and the reference disclosure are so small as to render the claims obvious over the reference. The features which the board found inadequately corroborated by appellant's evidence are the very features considered insufficient to patentably distinguish over the Harban reference. To hold that Harban is not removed by the showing here presented would lead to an anomalous result, i.e., if appellant broadened his claims by deleting the weight limita-

1. U. S. patent 3,197,453, isued July 27, 1965, on an application filed July 11, 1961.

2. An additional affidavit submitted by appellant's attorney stated that appellant had left the assignee's employ, was in a relatively inaccessible area of Peru, and hence was unavailable to execute the affidavit himself.

tions, so as to read literally on Harban, Harban would not be available as a reference against such broadened claims because appellant's antedating affidavit would be satisfactory in every respect.[3] It cannot be the law that the same affidavit is insufficient to remove the same reference applied against the slightly narrower claims presented here.

For the foregoing reasons, the board's rejection of the claims as unpatentable over Harban, under 35 U.S.C. § 103, is reversed.

Reversed.

58 CCPA

Application of Robert TOUVAY.
Patent Appeal No. 8370.

United States Court of Customs and Patent Appeals.

Jan. 14, 1971.

Appeal from Patent Office Board of Appeals' denial of claims Nos. 1–13, inclusive, of serial No. 318,421 for "manufacture of glass." The Court of Customs and Patent Appeals, Baldwin, J., held that claims were properly denied for obviousness.

Affirmed.

1. Patents ⇐18

Claims 1 to 13, inclusive, of patent application serial No. 318,421 for "manufacture of glass" were not patentable because of obviousness. 35 U.S.C.A. § 103.

2. Patents ⇐113(1)

On appeal from patent office's denial of claims because of obviousness, arguments directed to significance of particular limitation are not ordinarily considered unless they were raised below. 35 U.S.C.A. § 103.

3. Patents ⇐36(1)

Any failure of patentee of prior art to incorporate disclosures of much earlier patents was not evidence of unobviousness where record did not show either that prior art patentee actually knew of the other references or that he was seeking to solve a problem that was solved by applicant's invention. 35 U.S.C.A. § 103.

John L. Seymour, Bauer & Seymour, New York City, attorney of record, for appellant.

S. Wm. Cochran, Washington, D. C., for the Commissioner of Patents. Jere W. Sears, Washington, D. C., of counsel.

Before RICH, ALMOND, BALDWIN and LANE, Judges, and RE, Judge, United States Customs Court, sitting by designation.

BALDWIN, Judge.

Touvay seeks review of the decision of the Patent Office Board of Appeals which affirmed the examiner's rejection of claims 1–13, the only claims in his application,[1] as obvious in view of the prior art under 35 U.S.C. § 103.

THE INVENTION

Appellant's application discloses glass manufacturing apparatus of the type which includes generally

(1) a melting furnace heated by a series of combustion burners in the upper part thereof,

(2) discharge means for the furnace, including means for forming the molten glass into a ribbon, and

(3) a heated flotation chamber which receives the glass ribbon and

3. We recognize that, had appellant presented broader claims, the Patent Office might have found other, earlier art on which to reject them.

1. Serial No. 318,421, filed October 23, 1963, for "Manufacture of Glass".

Application of Lester L. SPILLER.
Patent Appeal No. 9174.

United States Court of Customs and Patent Appeals.
Aug. 8, 1974.
Rehearing Denied Nov. 14, 1974.

Claims for process of using electrostatic forces to apply dry starch particles to wet paper web and for the resulting cellulosic material were rejected by the Patent Office Board of Appeals, Serial No. 607,418, and claimant appealed. The Court of Customs and Patent Appeals, Rich, J., held that claimant had sufficiently shown reduction to practice of the claimed invention prior to major reference relied upon by Board of Appeals; that only the claims which related to sheets of paper coated with various amounts of starch were obvious; and that claims were not indefinite.

Affirmed in part and reversed in part.

1. Patents ⇐90(5)

In determining whether differences between the claimed invention and facts shown by the references and affidavits in support of prior reduction to practice are so small as to render the claims obvious, court may consider not only the knowledge of one skilled in the art but also the teachings of other references available as of the date of the alleged reduction to practice. 35 U.S.C.A. § 103.

2. Patents ⇐91(3)

Affidavits offered to remove a reference must establish possession of the invention and not merely possession of what the reference happens to show if that is wholly outside what is being claimed. Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

3. Patents ⇐90(5)

For purposes of antedating reference by showing prior reduction to practice, it is sufficient that claimant show reduction to practice of his basic invention, which showing will also suffice as to claims differing therefrom only in details which are obvious to one of ordinary skill in the art. 35 U.S.C.A. §§ 102, 103, 112; Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

4. Patents ⇐91(4)

Claims relating to the use of electrostatic forces to apply dry starch particles to wet paper web in order to form cellulosic sheet material, such as paper, had been reduced to practice prior to application for patent cited as reference and were valid. Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

5. Patents ⇐90(5)

Possession of what is shown by affidavits to have been reduced to practice carries with it possession of variations and adaptations which would, at the same time, had been obvious to one skilled in the art. 35 U.S.C.A. §§ 102, 103, 112; Patent Office Practice Rules, rule 131, 35 U.S.C.A. App.

6. Patents ⇐18

Claims for process of electrostatic deposition of dry starch on a wet paper web were not obvious in view of prior references. 35 U.S.C.A. § 103.

7. Patents ⇐18

Claims, which recited sheets of paper coated with various amounts of starch with the majority of starch particles being separated from one another on the paper surface, were obvious in view of prior references. 35 U.S.C.A. § 103.

8. Patents ⇐101(5)

Statutory requirement of definiteness in claim for patent is essentially requirement for precision and definiteness of claim language so that claims make clear what subject matter they encompass and thus what the patent precludes others from doing. 35 U.S.C.A. § 112.

9. Patents ⇐101(8)

There is nothing indefinite in use of claim language which defines particular amounts accord criterion. 35 U.S.C.A.

10. Patents ⇐101(11)

Claim for process with starch to modify was not indefinite mer ferred to coating "in a to be capable of causing cation of the surface U.S.C.A. § 112.

Arnold G. Gulko, Arl sler, Goldsmith, Clemen Chicago, Ill., attorney pellant; David H. B Corporation, Indianapo sel.

Joseph F. Nakamura C., for the Commissi Fred W. Sherling, Was counsel.

Before MARKEY, Ch LANE and MILLER, MOND, Senior Judge.

RICH, Judge.

This appeal is from the Patent Office Boar hered to on reconsider the rejection of claims der 35 U.S.C. § 103, claims 30–31 under 35 the rejection under 35 claims 1–2, 4–7, 9–11, a plication serial No. 607 ary 5, 1967, for "Manu and Similar Cellulosic M ified Surface Property Application of Dry Pow the Water-Wet Web B We reverse in part and

The Invent

The invention relates re of cellulosic sheet per which is coated w ove its surface prope ing of the paper is ctrically grounding th trostatically charging

ticular amounts according to functional criterion. 35 U.S.C.A. § 112.

10. Patents ⇐101(11)

Claim for process of coating paper with starch to modify surface properties was not indefinite merely because it referred to coating "in amounts sufficient to be capable of causing selective modification of the surface properties." 35 U.S.C.A. § 112.

Arnold G. Gulko, Arlington, Va., Dressler, Goldsmith, Clement & Gordon, Ltd., Chicago, Ill., attorney of record, for appellant; David H. Badger, Ransburg Corporation, Indianapolis, Ind., of counsel.

Joseph F. Nakamura, Washington, D. C., for the Commissioner of Patents. Fred W. Sherling, Washington, D. C., of counsel.

Before MARKEY, Chief Judge, RICH, LANE and MILLER, Judges, and ALMOND, Senior Judge.

RICH, Judge.

This appeal is from the decision of the Patent Office Board of Appeals, adhered to on reconsideration, affirming the rejection of claims 1–7 and 9–31 under 35 U.S.C. § 103, the rejection of claims 30–31 under 35 U.S.C. § 102, and the rejection under 35 U.S.C. § 112 of claims 1–2, 4–7, 9–11, and 19–23, of application serial No. 607,418, filed January 5, 1967, for "Manufacture of Paper and Similar Cellulosic Materials of Modified Surface Property by Electrostatic Application of Dry Powdered Starch to the Water-Wet Web Being Processed." We reverse in part and affirm in part.

The Invention

The invention relates to the manufacture of cellulosic sheet material such as paper which is coated with starch to improve its surface properties. Uniform coating of the paper is accomplished by electrically grounding the wet paper and electrostatically charging dry starch particles which are suspended in the atmosphere surrounding a water-wet web of paper, perhaps while the paper is still within a paper-making machine and on a Fourdrinier wire. This charge prevents the starch particles from contacting one another in the damp atmosphere near the wet web, and the electrostatic forces cause the starch to penetrate the air stream associated with the moving wet web and to gently attach themselves to the surface of the wet web.

The claims are numerous. The majority are directed to methods of manufacture of paper; claims 26–29 are allegedly directed to the resulting sheet of paper, 28 and 29 being specific to newsprint. Claims 30 and 31 are directed to the improvement in a conventional paper-making machine which comprises means for the electrostatic deposition of charged starch particles therein. Representative are claims 1, 26, 30, and 31.

1. A method of manufacturing a fibrous, absorbent, cellulosic sheet material, the steps comprising forming a water-wet web containing at least 25% by weight of water of fibrous cellulosic sheet material, depositing dry particles of starch upon said wet web by advancing said web past a particle deposition zone and supplying to said particle deposition zone said dry particles of starch electrostatically charged for mutual repulsion whereby said starch particles will be electrostatically attracted to and uniformly deposited upon said wet web in the form of separated particles and in amounts sufficient to be capable of causing selective modification of surface properties of the sheet material, and then dewatering said web.

26. A sheet of paper, said sheet having deposited on at least one side thereof starch particles in an amount of at least .02 pound of starch per 1000 square feet of surface, said starch particles being partially gelatinized in adherent association with the fibers on the surface of said paper and the majority of said starch par-

ticles being separated from one another on said paper.

30. In a paper-making machine the improvement which comprises means for advancing a wet paper web past a particle deposition zone, means for supplying to said particle deposition zone electrostatically charged particles of starch whereby such charged particles will be attracted to said paper web in the form of separate particles and uniformly deposited thereon.

31. Apparatus as recited in claim 30 in which said particle deposition zone is positioned above the free upper surface of the paper as it is carried by said advancing means constituted by a Fourdrinier wire.

The References and the Rejections

The references are:

Uong	2,030,483	Feb. 11, 1936
Read et al. (Read)	3,210,240	Oct. 5, 1965
Lichtenberger et al. (Lichtenberger) (Effective filing date Nov. 24, 1965)	3,461,032	Aug. 12, 1969
Smith et al. (Canadian) (Smith)	704,036	Feb. 16, 1965

Casey, Pulp and Paper, 2nd Ed., N. Y. Interscience, 1960, page 951.

Reif, "An Electrostatic Process for Applying Dry Coatings on Paper," TAPPI, October 1955, Volume 38, No. 10, pages 607–609.

The board having affirmed the rejection of claims and groups of claims on three different statutory bases, 35 U.S.C. §§ 102, 103, and 112, we shall deal with them individually.

The Rejections Applying Lichtenberger

A principal issue is whether appellant's affidavit showing under Rule 131 is sufficient to remove Lichtenberger as a reference. Appellant admitted explicitly at oral argument, and implicitly in his briefs here and before the board, that the various rejections under § 102 and § 103 which use Lichtenberger alone or in combination with other references were proper, and it was therefore essential to overcoming them that he remove Lichtenberger as a reference. Such rejections are applicable against all claims.

Lichtenberger is used as a reference in various ways. First, as against apparatus claims 30 and 31, which describe an improvement in a paper-making machine, Lichtenberger is applied alone under § 102, allegedly identically disclosing the invention. Secondly, Lichtenberger is applied alone under § 103 to claims 1, 2, 5, 9, 10, 11, and 19–23. Finally, all the other claims are rejected under § 103 in view of various combinations of Lichtenberger and other references, Lichtenberger and Smith to render obvious the invention of claims 3, 12, 13, 15–18, and 24–28, and additionally with Casey as to claims 4, 6, 7, 14, and 29. We have noted the various claims and the ways in which they were rejected on Lichtenberger alone, or in combination with other references, because of the differences which appear in this regard between this case and the prior Rule 131 cases in this court upon which the decision in this case is to rest.

The Rule 131 Evidence

The inventor Spiller's affidavit and evidentiary material submitted therewith, including laboratory notebook pages and accompanying affidavit of Spiller's associate, Stephen J. Smith, establish that Spiller and his associate performed certain acts which, in his view, establish a reduction to practice of the claimed invention prior to the earliest effective filing date of Lichtenberger, November 24, 1965. Grounded wet TAPPI [1] blotting paper was moved over a fluid bed of powdered starch electrostatically charged to a level of 20–40 kilovolts. The starch was electrostatically propelled into contact with and adherently deposited on the surface of the wet paper, which had been pretreated with a solution of potassium iodide and iodine to color the deposited starch particles so

1. TAPPI is the acronym of the Technical Association of the Pulp and Paper Industry.

that they could be seen and the uniformity of the deposit thus observed. The paper sheet was oven dried and weighed prior to deposit of the starch and the wet paper with the starch deposit thereon was dried after the deposition to gelatinize the wet starch on the wet paper and redry the paper for subsequent weigning to determine the amount of starch deposited on the paper. The Smith notebook pages establish the various weight amounts of starch deposited on the paper at various voltages of electrostatic charge. Smith noted, "Works very well & can put as much on as we want." And also, "The higher the voltage the better the coverage."

Also in evidence is a short letter to appellant from L. S. Simser of August 12, 1965, who represented Penick & Ford Limited, of Cedar Rapids, Iowa, the company which supplied the starch and TAPPI blotting paper to appellant, and a portion of a letter from appellant's patent counsel, A. G. Gulko, dated October 14, 1965, which discusses appellant's invention. The latter apparently reported the results of a prior art search made on the invention (report not of record) and states Mr. Gulko's view of the invention as follows:

> As I understand the present development, finely divided soluble starch powder is deposited by means of electrostatic forces from a fluid bed onto the surface of wet paper. The fluid bed underlies the wet paper as it goes through the paper-making process where the wet paper contains between about 40% to 70% by weight of water.

Spiller's affidavit refers to the Gulko letter as helping to record the details of the "demonstration" relied upon for a reduction to practice. While various other facts shown by the affidavits or the Smith notebook pages will be discussed later, we will start from the proposition that appellant's affidavits and evidence establish electrostatic coating of dry starch particles on re-wet TAPPI blotting paper.

The Opinion of the Board

The opinion of the board and the Examiner's Answer, with which the board agreed, list and discuss various *elements* of the thirty claims which are believed not to have been established by the affidavit showing. The examiner stated, in pertinent part (emphasis ours):

> The affidavits and exhibits do not show the *amounts* of claims 3, 12–18 and 24–29, nor the *water contents* of claims 1–25. There is no showing of any *apparatus* remotely like that of claims 30 and 31. There has been no allegation or showing that the starch was applied to a web containing *at least 25% moisture*. The affidavits and exhibits do not show a process or apparatus such as that *claimed*. Thus the affidavit fails to prove the *heart of the claimed invention*, ie, application of particles to a wet web. The affidavits do not prove that the process was done in conjunction with a papermaking machine nor that the apparatus was part of a papermaking machine.

The board added the following criticism of its own:

> There is no clear evidence of record that Appellant's technique employing wet TAPPI blotting paper is an industry-*recognized* test duplicative of wet web on a Fourdrinier wire. The notebook exhibits, describing work done some 4½ years prior to the affidavits, do not make it apparent that the starch deposited from a fluidized bed was *dry*, or that the paper was *wet*, or that there was any dewatering step, or indeed that the starch particles were "electrostatically charged for mutual repulsion" and "separated from one another," all as required by the claims. Nor is any ratio of starch to paper surface indicated, or any surface property mentioned which might have been modified by the treatment. In connection with the rejections of claims 17, 18, 25, 28, 29 and 31, note that the Rule 131 showing concerns

only depositing starch *underneath* the paper. Lichtenberger et al. stands as a valid reference.

And, in addition, the board incorporated in its opinion by reference the following portion of an opinion by another panel of the Board of Appeals in a copending application [2] of appellant where the merits of the same Rule 131 evidence were discussed:

> Considering the merits of the Rule 131 evidence, affiant Spiller, in his affidavit executed January 13, 1970, states that "TAPPI blotting paper, when wet, duplicates the condition of wet paper on a Fourdrinier machine." There is nothing to correlate this 1970 observation with the data recorded in the Smith notebook bearing dates in August and September 1965. The fragment of a letter appearing on the letterhead of Arnold G. Gulko refers to applying starch powder to "the wet paper as it goes through the paper-making process where the wet paper contains between about 40% to 70% by weight of water." Again there is no correlation between this observation and the Smith notebook entries. It would appear that appellant is attempting to create the illusion that the Smith laboratory work had something to do with the electrostatic deposition of dry starch in a wet web of paper on a paper-making machine, which concept is totally lacking in the Smith notebook entries but fully disclosed in Lichtenberger et al.
>
> At best, it would appear that Smith "shot" starch from a fluidized bed onto pieces of blotting paper of unspecified dimension and of unspecified, if any, water content. Such pieces of blotting paper hardly qualify as a "water-wet web" as required by the claims on appeal and as disclosed in Lichtenberger et al. Certainly if the water content of the blotting paper was of significance with respect to starch deposition, this circumstance was unobserved by Smith. The Spiller affidavit states that "Both visual observation and microscopic observation demonstrated that the deposit was at the surface and uniform" but there is no evidence in this record concerning who made such observations or when they were made. There is nothing in the Smith notebook which indicates that any of the samples prepared by Smith were inspected.

Appellant's Arguments

On the sufficiency of the affidavit showing, appellant's brief individually attacks each reason given by the examiner and both boards as to why the showing made by appellant does not satisfy each limitation of the claims. For example, in answer to the specific allegation that the acts established by the affidavit showing do not include the use of a water-wet web containing at least 25% moisture, which is a limitation of all method claims 1–7 and 9–25, appellant states that "the mere characterization of paper as 'wet' [which is what is established by the Spiller affidavit and Smith notebook] roughly indicates at least this amount of water." In addition, appellant maintains that the quotation of the contemporary letter from counsel "indicates that the work done was estimated to have been done with paper wet with: '. . . between about 40% to 70% by weight of water.' With respect to the board's comment that the failure of the affidavit showing to establish "any ratio of starch to paper surface," called for by many dependent claims, appellant notes only that none of the claims (1, 2, 4–11, 19–23, 30 and 31) do not specify the ratio of starch to paper surface. And as to the board's criticism of the appropriateness

2. Application of Lester L. Spiller, serial No. 607,511, for "Manufacture of Paper and Similar Cellulosic Materials of Modified Surface Property by Electrostatic Application of Dry Powdered Water-Sensitive Re... the Water-Wet Web Being Proce... board opinion of April 30, 1971.

TAPPI blotting paper as representative of the condition of the wet web of paper on the Fourdrinier paper-making machine, appellant notes that Spiller's affidavit specifically alleges that "TAPPI blotting paper, when wet, duplicates the condition of wet paper on a Fourdrinier machine." Appellant further states that blotting paper is merely a web of uncompacted paper fibers so that when it is wet it is then a water-wet web, and, accordingly, the only difference between it and the web on a paper-making machine is perhaps that the latter is continuous, and the blotting paper is of a specific length.

The Prior Cases

The problem with the characterization of the Rule 131 sufficiency issue by the examiner and the two boards is that it is nowhere stated just what appellant must show in order to antedate Lichtenberger. The examiner first stated that "The affidavits and exhibits do not show a process or apparatus such as that claimed," thus implying that it is necessary to show a reduction to practice of the invention as it is claimed, presumably in each and every claim. Then the examiner states that, "Thus the affidavit fails to prove the *heart of the claimed invention*, ie, application of particles to a wet web." (Emphasis ours.) But what is the "heart" of the claimed invention, and how is it to be determined when there are many different claims?

As noted earlier, appellant's brief attempts to refute each factual statement of the examiner and the board panels, but, like them, never cites any standard for what must be shown to antedate Lichtenberger, in relation either to what Lichtenberger shows or to what is claimed. The solicitor's brief, for the first time in the prosecution of this application, states what he believes the decisions of this court suggest a patent applicant must show to antedate a reference, saying:

It is submitted that appellant has failed to show "priority with respect to so much of the claimed invention as the reference happens to show." In re Stempel, 241 F.2d 755, 44 CCPA 820 [1957]. Also, appellant has failed to show prior "possession of either the *whole* invention claimed or something falling *within* the claim, in the sense that the claim as a whole reads on it." In re Tanczyn, 347 F.2d 830, 52 CCPA 1630 [1965].

Stempel and *Tanczyn* at least lay a foundation for determining the sufficiency of the Rule 131 showing, but how are the two quoted statements applicable to the situation here? If what the reference shows is the important factor, no one has delineated what this is and whether the Rule 131 affidavits show it, particularly since the "claimed invention" varies with the individual claims. If apparatus claims 30 and 31, referring to "a paper-making machine," represent the claimed invention, then, at least according to the admittedly correct examiner's rejection of these claims, the reference shows the invention. If, however, the invention is taken to be what is claimed in the method claims, Lichtenberger may not specifically show the invention but may render it obvious, at least according to the § 103 rejections made in view of Lichtenberger alone, or in view of one or more other references.

Taking what the affidavits and other evidence show as having been performed, again great variances exist for individual claims. Asking whether claims 30 and 31, in the words of *Tanczyn*, "read on" what appellant did prior to the effective date of the reference, might require a negative response because of the fact that appellant's demonstration used no Fourdrinier paper-making machine. In this sense, appellant shows less than what the reference shows. Using method claim 1, supra, as representative of the invention, however, the answer to the question whether this claim "reads on" appellant's prior activities may depend on whether it is estab-

lished that appellant's paper contained "at least 25% by weight of water."

Resolution of the question of the sufficiency of the Rule 131 showing resides, at least in part, in decisions of this court after *Tanczyn*, not cited by either party, which dealt with fact situations where the showing made by Rule 131 affidavits was less that the invention *claimed* but was held sufficient to remove the cited reference because the differences were obvious. In re Hostettler, 356 F.2d 562, 53 CCPA 1069 (1966); and In re Stryker, 435 F.2d 1340, 58 CCPA 797 (1971).

In *Stryker*, we determined the sufficiency of a Rule 131 showing which established all of the *claimed* invention *except* for specific weight percentage limitations. We noted that appellant's showing was commensurate with that of a Harban reference, which had been solely relied upon by the Patent Office to render the claimed invention obvious, and that it was the specific weight percentages which appellant's affidavit evidence failed to establish which were considered by the Patent Office to be obvious in view of the reference. We noted that the "differences between the claimed invention and the reference disclosure [as well as appellant's Rule 131 showing] are so small as to render the claims obvious over the reference [and over appellant's showing]," and held the showing sufficient to antedate the reference. The board in *Stryker*, like the examiner here, had stated the proposition, for which *Tanczyn* was cited, that "The *claimed* invention must be shown in the affidavit, i. e., the [weight percentage limitations]." We held that this was not necessary, saying:

> To hold that Harban is not removed by the showing here presented would lead to an anomalous result, i. e., if appellant broadened his claims by deleting the weight limitations, so as to read literally on Harban, Harban would not be available as a reference against such broadened claims because appellant's antedating affidavit would be satisfactory in every respect. It cannot be the law that the same affidavit is insufficient to remove the same reference applied against the slightly narrower claims presented here. [Footnote omitted.]

The situation here with respect to the invention of, for example, claim 1, is much like that in *Stryker*. The original rejection by the Patent Office was for obviousness over a single reference, Lichtenberger. Appellant's showing seems to be commensurate with the showing of the reference, but, as noted by the examiner and by the board in the related application, neither expressly shows the limitation of the claim that the wet web contains "at least 25% by weight of water."[3] This is like the reference disclosure and affidavit showing in *Stryker* which failed to show the weight percentage limitations of Stryker's claims.

[1, 2] Many dependent claims also raise the question whether the situation in *Stryker*, where the differences between the claimed invention and what the reference and affidavits showed were "so small as to render the claims obvious" to one skilled in the art in view of a single reference, ought to be extended to a situation where part or all of the differences are rendered obvious not merely by the knowledge of one

3. Lichtenberger's web, like that in appellant's showing, is taught to be wet; and both *may in fact* contain the minimum quantity of water claimed. Lichtenberger does not specifically teach this, however, and we do not find that the water quantity has been established by appellant's evidence. There is nothing in the Gulko letter to tie the water percentage figure mentioned to the *demonstrations* relied upon for reduction to practice. The water percentage figure could just as well have come from appellant's conception of the invention as from the demonstrations. However, we do think appellant established that his web was "wet" during the demonstrations. Appellant's blotting paper web is clearly shown by the Smith notebook to have been electrically "grounded," and a wet starch indicator (potassium iodide and iodine solution) was used thereon.

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skilled in the art but by *other references* available as of the date of the alleged reduction to practice. This court has recently answered that question in the affirmative, at least when a single other reference is involved, in In re Dardick, 396 F.2d 1234 (CCPA 1974), and we see no reason to distinguish the situation where two other references are used. As we noted in *Dardick*, the reference —or the references—are merely used to show what one skilled in the art would be expected to know as of the date of the reference which has been removed. The only additional caveat which ought to be added is that the affidavit showing must still establish possession *of the invention* and not just of what a reference happens to show if this is "wholly outside" what is being claimed. In re Tanczyn, supra.

[3] The invention embodied in claims 30 and 31 raises an additional difference between this case and the situation in *Stryker*. In *Stryker*, we said that "the differences . . . are so small as to render the claims obvious over the reference," and Stryker's Rule 131 showing was commensurate with the reference. Here, taking as the invention and embodiment of claims 30 and 31, appellant's showing may well render the invention as there claimed obvious, but it does not appear to establish possession of all the limitations of these claims since appellant's demonstration did not include the use of his electrostatic deposition technique on a Fourdrinier papermaking machine. The question, then, is whether the rule of *Stryker* ought to be extended to a situation where the Rule 131 showing is not fully commensurate with the reference but renders the claimed invention obvious. We think *Stryker* is controlling in this situation as well, and that such an extension of *Stryker* is supported by our earlier decision in *Hostettler*, supra.

In *Hostettler*, we considered the sufficiency of a Rule 131 showing under conditions similar to the present situation as it relates to claims 30 and 31. The invention was a catalytic process for producing urethane. The reference, which admittedly anticipated the invention, showed the claimed process as producing *poly*urethane, while the Rule 131 showing established the use of the catalyst only to produce a *monomeric* urethane compound. Our opinion characterized the invention as "the use of a catalyst in a process involving an old reaction" and held the showing sufficient, although relating to an embodiment of the invention *different* from that shown by the reference. Using a prior art Rothrock patent, cited during the prosecution, as indicative of the knowledge of one in the art, we concluded that "one of ordinary skill in the art would be satisfied from the facts shown in the affidavit that appellants had completed the invention as defined in the claims." We stated:

> It is clear on this record that one of ordinary skill in this art would consider that functionality of the reactants determines whether the products are "monomeric" or polymeric, but not that functionality would matter insofar as the reaction using the catalyst is concerned. The statement as to unpredictability of catalytic activity [by the examiner], while relevant, is so general as to afford little assistance in the determination of the precise issue before us. In fact, the more specific showings in the affidavit and the Rothrock patent indicate that one of ordinary skill in this art would expect that a catalyst for the particular functional groups involved in the reaction would operate relatively independently of the number of those groups on the reactant molecule. Thus we conclude that one of ordinary skill in the art would be satisfied from the facts shown in the affidavit that appellants had completed *the invention* as defined in the claims. See In re Fong, supra [288 F.2d 932, 48 C.C.P.A. 897 (1960)]. Certainly appellants should not be required to submit facts under

> Rule 131 showing that they reduced to practice that which is obvious in addition to those facts offered as showing a completion of the invention, for the purposes of antedating a reference. [Footnote omitted.]

The *Fong* case cited in the above quotation is significant here. We believe that similar considerations govern our decision and that, for the purpose of antedating the Lichtenberger reference under Rule 131, it is sufficient that appellant has shown a reduction to practice of his basic invention, which showing will also suffice as to claims differing therefrom only in details which are obvious to one of ordinary skill in the art.

[4] The heart of this invention is clearly the use of *electrostatic forces* to apply *dry starch* particles to a *wet paper web*. The differences which exist between the claimed invention and the specific reduction to practice established by the Rule 131 showing are such as would be obvious to one of ordinary skill in this art. In re Stryker, supra; In re Dardick, supra; In re Hostettler, supra; In re Fong, supra.

We are satisfied that the differences in the embodiments called for by the claims are so small that the claimed invention would have been obvious. Taking the claims in numerical order, claim 1, and the claims dependent thereon, call for a moisture content of "at least 25% by weight of water" in the web. While we are not satisfied that the Rule 131 showing establishes this content *in the reduction to practice*,[4] we are satisfied that, at the least, one skilled in this art would find it obvious to have an admittedly "wet" web contain such an amount of moisture and that wet blotting paper would be considered to be a "water wet web."

With respect to limitations of the claims which require specific ratios of starch to paper surface and the modification of surface properties by the deposition of starch on the paper, we are satisfied that these limitations too would be obvious to one skilled in the art familiar with the facts shown by the Spiller affidavit and the Smith notebook entries. Certainly it was well known that starch modified the surface properties of paper generally. It was no part of applicant's invention to apply starch to paper. He merely applied it by a new technique. The fact that *several* different weights of starch were applied to the paper by various numbers of passes and with various values of voltage would render the deposition of specific amounts which are claimed obvious.

[5] With respect to the deposition of starch on both sides of the paper, called for by claims 17, 18, 28, 29 and 31 which the board found unsupported by the Rule 131 showing of "only depositing starch *underneath* the paper," this and the other differences called for by the claims over what was reduced to practice would clearly be obvious to one of ordinary skill in this art.[5]

The differences between the Rule 131 showing and the invention as it is described in claims 30 and 31, which call for using the electrostatic starch deposition technique in a paper-making machine and on the Fourdrinier wire thereof, are also obvious differences. While appellant's argument, and consequently the Patent Office response thereto, centered on the equivalence or duplication of the conditions of the wet paper on the Fourdrinier machine by the use of TAPPI blotting paper, we think that the obviousness of the use of the technique on the paper-making machine, as claimed, is clear. Spiller's affidavit states that

4. See note 3, supra.

5. Since the purpose of the Rule 131 showing is to establish broadly possession of the invention, In re Tanczyn, supra, it is proper to consider the obviousness *of the differences* between what is shown and what is claimed because possession of what is shown carries with it possession of variations and adaptations which would, at the same time, be obvious to one skilled in the art.

"TAPPI blotting paper, when wet, duplicates the condition of wet paper on a Fourdrinier machine," and the contemporaneous letter of Simser made reference to the use of electrostatic coating "in the paper industry." If there is any doubt as to the knowledge of one skilled in the art, it is clear from the Uong reference that the art had long known of depositing dry coatings directly upon the water-wet paper web on the Fourdrinier wire in a paper-making machine.

Accordingly, the rejections under § 102 and § 103 in view of Lichtenberger and Lichtenberger in combination with other references are reversed.

The Prior Art Rejections Exclusive of Lichtenberger

[6] We have studied the five references variously applied by the Patent Office under § 103 and have concluded that they do not render obvious the *electrostatic* deposition of dry starch on a wet paper web. The only reference which deals with electrostatic coating is Reif, which discloses coating finely divided powders on a dry paper web. The solicitor notes correctly that "Reif does not [explicitly] disclose whether the paper being coated is wet or dry." However, it is quite clear from the Reif disclosure that the paper is not at all wet. Reif states:

> Web speed probably will be limited only by the rate at which the electrical charge can leak off the powder deposited on the web. The electrical charges must drain off the coated web before leaving the strong electrostatic field. Otherwise, repulsion of the like charges on the web and coating powder may cause disturbances in the coating that will mar the appearance of the finished paper.
>
> Once the coating is deposited on the paper and leaves the coating unit, it is fixed to the paper with heat. This fusing is done as the web passes through an infrared oven or over a heated roll. Had the paper been wet, there would be no need to drain the charge off the coated web to avoid disturbances in the coating before the application of heat to fix the coating to the paper. Moreover, we cannot accept the argument of the solicitor that "dry paper contains a substantial amount of moisture" as a teaching that the paper may be wet.

In addition, we can find no reason why it would be obvious to one skilled in the art to combine the Reif teachings of using electrostatic forces in paper coating with the teachings of the other references, which suggest coating paper with starch in either wet or dry form. The wet application of starch is shown by Read, Smith, and Casey and electrostatic forces would be useless in combination therewith. Uong does teach the application of powdered coating materials to the wet web of paper in a paper-making machine, but we do not believe that it would have been obvious to one of ordinary skill in the art to combine electrostatic force therewith. We agree with appellant that the use of starch particles in the Uong enclosure above the wet web would probably result in the formation of starch agglomerates and make the process inoperable. The solicitor's answer (to appellant's assertion) that "it would be all the more obvious to electrostatically charge the particles as taught by Reif since the well-known advantage of electrostatic coating is the prevention of agglomeration" is a hindsight application of appellant's teachings. Reif does not teach such an advantage and, using a dry insulated sheet and not starch, never had such a problem to overcome.

Accordingly, the rejections under 35 U.S.C. § 103 of claims 1–7, 9–25, and 30–31 are reversed.

[7] As to the four claims 26–29, which recite sheets of paper coated with various amounts of starch with "the majority of said starch particles being separated from one another on said" paper surface, we agree with the solicitor that "appellant has failed to show that the product is unobviously different from that of Read or Smith." These claims are not limited to the starch coated pa-

per produced through electrostatic coating of wet paper, and we do not believe that they have been shown to sufficiently distinguish over the art by the properties of the starch on the paper and such limitations as that above-quoted. The rejections of claims 26–29 under § 103 are affirmed.

Section 112, Indefiniteness

The board affirmed the rejection of claims 1, 2, 4–7, 9–11, and 19–23 under the second paragraph of § 112 for the reasons given by the examiner, which are:

> Applicant has failed to recite the amount of starch applied to the web in quantitative terms or in definite qualitative terms. Applicant relies on the phrase "in amounts sufficient to be capable of causing selective modification of surface properties". It is not even clear whether the modification will improve the properties or what properties will be modified. These properties could range from pick resistance, smoothness, sizing, wet strength, color to the taste. Furthermore, it is not clear whether applicants' claims are meant to include the application of two or three particles, because two or three particles would selectively modify the surface properties. Likewise a coating of starch one inch thick on the surface of the web would also modify the surface.

[8, 9] We recently restated what is meant by the "indefiniteness" requirement of § 112. It is essentially a requirement for *precision and definiteness* of claim language so that the claims make clear what subject matter they encompass and thus what the patent precludes others from doing. In re Conley, 490 F.2d 972 (CCPA 1974). Taking the requirement as such, we find that there is no indefiniteness in the use of the above-quoted language in the rejected claims. There is nothing indefinite in the use of claim language which defines particular amounts according to a functional criterion. See In re Fuetterer, 319 F.2d 259, 50 CCPA 1453 (1963); In re Swinehart, 439 F.2d 210, 58 CCPA 1027 (1971).

[10] The examiner's statement that "It is not even clear whether the modification will improve the properties," and his extreme example that "a coating of starch one inch thick on the surface of the web would also modify the surface" almost suggest that there is something sinister in appellant's claiming something which would not, *in the examiner's view*, "improve" the properties of the paper coated. As far as the rejection of the claim language for idefiniteness is concerned, we think that the claims make it clear, through the use of the word "selective," that any modification of surface properties is subjectively desirable in the particular coating applied. Even a "selective" modification of surface properties through the application of an inch of starch to the coated paper —absurd though that would be—would be within the language of the claims. Thus the claim language makes clear what subject matter they encompass.

In In re Conley, supra, we dealt with, and reversed, a similar rejection of claims as indefinite for failure to recite specific proportions and amounts of certain ingredients, one of which was the proportion of satin white in an aqueous suspension. Appellants in *Conley* maintained that there were "no critical proportions of satin white in aqueous suspension." We note that here also there is no criticality in the amount of starch which is to "be electrostatically attracted to and uniformly deposited upon" the wet web, and we agree with appellant that there is no reason why he must state in his claims "a feature of no importance" to his invention, which, as previously noted, is the electrostatic deposition of dry starch on wet paper. As appellant's brief points out, the original reduction to practice of the invention which appears from the Rule 131 affi

vit evidence, shows that from the very beginning it was determined by appellant's associate that, with respect to the amount of starch deposited, we "can put on as much as we want." And appellant's specification states that:

> In practicing the present invention, the amount of starch applied can be varied considerably while still achieving some of the benefits of the invention.

Accordingly, we find no indefiniteness in defining the amount of starch. The rejection of claims 1, 2, 4–7, 9–11, and 19–23 for indefiniteness is reversed.

Summary

In accordance with the foregoing, the rejections of claims 1–7, 8–25, and 30–31 are reversed, and the rejection of claims 26–29 under § 103 is affirmed.

Modified.

evidence directed to Schwartz's state of health of record before the PTO is not to the contrary. Schwartz himself told Mrs. Lonardo in the March 24, 1974, telephone conversation that he had been ill and he had neglected his work. Mrs. Lonardo heard in 1974 from another attorney in Rhode Island, Elliot Salter, that Schwartz had been ill "for some time." Leonard Michaelson, also an attorney in Rhode Island, testified that Schwartz had had a heart attack ten years or so before his death.

Based on the findings above, one would anticipate that if Schwartz continued his patent practice following April 1973, he would begin to fail in his professional duties, and that such failures will become more numerous as time went on. Indeed, the facts discussed below are in accordance with that anticipation. In particular, with regard to nine filed applications including the '365 application, Schwartz failed in his responsibilities once in 1974, once in 1976, once in 1977, once in 1978, thrice in 1979, and twice in 1980.

The prosecution history of seven other applications prosecuted by Schwartz from the period of June 1976 to December 1980, are relevant. Those applications, in chronological order of the filing date, are:

	Serial No.	Filing Date	Pa tent No.
1.	* * * *	* * * *	(not issued)
2.	696,486	06/15/76	4,378,948
3.	852,082	11/16/77	4,356,793
4.	* * * *	* * * *	(not issued)
5.	D 949,812	10/10/78	D.269,300
6.	D 949,813	10/10/78	D.268,619
7.	D 19,460	03/12/79	4,545,378

Each of the above-identified seven applications became abandoned sometime during prosecution as a result of Schwartz's failure either to respond at all or to respond timely to an office action. Applications 1 and 4 above are not specifically identified because they have not issued as United States patents and thus have confidential status under 35 U.S.C. §122. Schwartz refiled applications 3, 5 and 6 in December 1980, even though there were intervening sales in at least application 3.

Application 1 became abandoned because Schwartz did not respond to an office action dated October 21, 1977, for which a response was due on December 21, 1977. Application 2 became abandoned because Schwartz did not respond to an office action dated September 22, 1976, for which a response was due on December 22, 1976. Application 3 be came abandoned because Schwartz did not respond to an office action dated September 26, 1978, for which a response was due on December 26, 1978. Application 4 became abandoned because Schwartz did not respond to an office action dated March 27, 1980, for which a response was due on June 27, 1980. Application 5 became abandoned because Schwartz did not respond to an office action dated July 25, 1979, for which a response was due on August 25, 1979. Application 6 became abandoned because Schwartz did not respond to an office action dated June 4, 1979, for which a response was due on July 5, 1979. Application 7 became abandoned because Schwartz filed a response on January 28, 1980, to an office action dated October 25, 1979, for which a response was due on January 25, 1980.

An eighth application prosecuted by Schwartz, serial number 912,385, filed on June 5, 1978, also became abandoned as a result of Schwartz's failing to respond to an office action dated October 25, 1978, for which a response was due on January 25, 1979. Schwartz succeeded in reviving the abandoned application under Rule 137 on the basis of a mistake in docketing the office action for response; his petition to revive the application was granted on November 28, 1979. That application is now issued as United States Patent No. 4,211,190.

After Schwartz's death, petitions were filed in each of the above-listed seven applications to have them revived. The respective petitions were followed by a consolidated petition for revival of all seven applications. In all applications except for applications 2 and 5, the initial petitions had already been denied when the consolidated petition was filed. Subsequent to the filing of the consolidated petition in each application, the petitions were granted and each application was revived. In each decision granting respective petitions, the PTO attributed Schwartz's failure to respond timely to his "inability to perform his responsibilities."

The seven applications were revived mainly on the basis of the consolidated petition, which included (1) a declaration of Dr. Ezra A. Sharp; and (2) a declaration of Herbert Barlow, a patent attorney who took over several of Schwartz's on-going patent matters after Schwartz's death. Incidentally, it is noted that the consolidated petition misstated the filing date of application 1 as January 21, 1978, of application 2 as December 22, 1976, and of application 7 as January 25, 1980.

In addition to Dr. Sharp's testimony already discussed above, Dr. Sharp stated:

> In recent years I have had no doctor patient relationship with Max Schwartz that would enable me to provide a professional opinion as to his mental deterioration in recent years. However, his senility would not be inconsistent with my prior observations of him during those occasions when I was called upon to treat his heart problems.

Mr. Barlow stated that his law firm assumed the prosecution of a number of patent applications which were formerly handled by Schwartz. His testimony recounted three instances in which Schwartz had not filed completed United States patent applications which should have been filed, and nine instances in which Schwartz caused erroneously instructed foreign associates to drop the prosecution of corresponding foreign applications. Mr. Barlow stated that the foreign applications were filed "in the fall and early spring of 1978–79." He also stated that one of the three unfiled United States patent applications included a signed declaration dated September of 1979; no dates for the other two unfiled United States applications were noted.

[2] As evidenced above, Schwartz's course of professional failures subsequent to April 1973 was progressively worse. The failures began in early 1974 and became more frequent in the following years. Because Schwartz's state of health became precarious as early as April 1973, there is no reason to isolate the year 1974 and treat it differently from the later years. Accordingly, the initial abandonment of the '365 application was due at least in part to Schwartz's illness and thus excused within the meaning of unavoidable delay under 35 U.S.C. §133. *See e.g. In re Mattullath*, 1912 Dec. Comm'r Pat. 490, 493 (App. D.C. 1912); *Ex parte Sellers*, 1905 Dec. Comm'r Pat. 336 (Comm'r Pat. 1905); *McDuffee v. Hestonville*, 181 F. 503, 510–11 (E.D. Pa. 1910).

Conclusion

For the foregoing reasons and on this rather unusual set of facts, Lonardo has demonstrated unavoidable delay within the meaning of 35 U.S.C. §133, and the renewed petition under 37 CFR §1.137(a) to revive the '365 application from abandonment is *granted*.

U.S. Patent and Trademark Office
Board of Patent Appeals and Interferences

Ex parte Levy

No. 90-1864

Decided October 16, 1990
Released November 8, 1990

PATENTS

1. Patentability/Validity — Anticipation — Identity of elements (§115.0704)

Factual determination of anticipation requires disclosure in single reference of every element of claimed invention, and examiner must identify wherein each and every facet of claimed invention is disclosed in applied reference.

2. Patentability/Validity — In general (§115.01)

Patentability/Validity — Anticipation — Prior art (§115.0703)

Initial burden of establishing prima facie basis to deny patentability rests upon examiner; examiner, if relying upon theory of inherency, must provide basis in fact and/or technical reasoning to reasonably support determination that allegedly inherent characteristic necessarily flows from teachings of applied prior art.

3. Patentability/Validity — Anticipation — Prior art (§115.0703)

Examiner erred by rejecting claims for biaxially oriented catheter balloon as anticipated by prior art which does not disclose such biaxially oriented balloon and which has not been shown to be inherently biaxially oriented.

4. Patentability/Validity — Obviousness — Relevant prior art — Particular inventions (§115.0903.03)

Examiner erred by rejecting claims for biaxially oriented balloon catheter under 35 USC 103 based upon combined disclosure of two prior art references, one of which was relied upon solely for disclosed use of high viscosity polyethylene terephthalate tubing and the other which was presupposed by examiner to disclose biaxially oriented catheter balloon, since examiner has not established that resulting catheter balloon using high viscosity tubing is biaxially oriented.

Application of Stanley B. Levy, serial no. 287,234, filed Dec. 21, 1988, which is a division of serial no. 914,108, filed Oct. 1, 1986, now Re. 32,983, granted July 4, 1989; and a reissue of serial no. 510,812, filed July 5, 1983, now patent no. 4,490,421, granted Dec. 25, 1984, for balloon and manufacture thereof. From examiner's rejection of claims 13 through 17 and 25 (James Seidleck, pri-

mary examiner), applicant appeals. Reversed.

Louis H. Rombach, Wilmington, Del., for appellant.

Before Steiner, Tarring, and J. Smith, examiners-in-chief.

Steiner, examiner-in-chief.

This is an appeal from the final rejection of claims 13 through 17 and 25, which are all of the claims remaining in this application for reissue of U.S. Patent No. 4,490,421.

The subject matter on appeal is directed to a polymeric balloon exhibiting properties which enable its use as a catheter balloon for medical dilation procedures, such as coronary angioplasty wherein a catheter with a balloon at a distal end thereof is inserted into coronary arteries and inflated. The balloon must be capable of exerting sufficient pressure to dilate stenotic lesions without rupture of the balloon.

Claims 13 and 25, the only independent claims on appeal, read as follows:

13. *High molecular weight, biaxially oriented, flexible polymeric balloon having a wall tensile strength of at least 31,714 psi (218.86 MPa).*

25. *High molecular weight, biaxially oriented, flexible polyethylene terephthalate dilatation catheter balloon.*

The references relied upon by the examiner are:

Wyeth et al. (Wyeth)	3,733,309	May 15, 1973
Schjeldahl et al. (Schjeldahl '989)	4,413,989	Nov. 8, 1983 [1]
Schjeldahl et al. (Schjeldahl '000)	4,456,00	June 26, 1984 [2]

Claims 13, 14, 16, 17 and 25 stand rejected under 35 U.S.C. 102 as anticipated by Schjeldahl. Claims 13 through 17 stand rejected under 35 U.S.C. 103 based upon "Schjeldahl et al in view of Wyeth as set forth in the Final Rejection" (paragraph bridging pages 3 and 4 of the Answer). We reverse each rejection.

[1] Each of the Schjeldahl references contains essentially the same relevant disclosure. Accordingly, unless otherwise indicated, we have referred to these references collectively as "Schjeldahl," consistent with the approach adopted by both appellant and the examiner.

[2] See footnote 1.

The Rejection of Claims 13, 14, 16, 17 and 25 Under 35 U.S.C. §102.

[1] The factual determination of anticipation requires the disclosure in a single reference of every element of the claimed invention. *In re Spada,* ___ F.2d ___, 15 USPQ2d 1655 (Fed. Cir. 1990); *In re Bond,* ___ F.2d ___, 15 USPQ2d 1566 (Fed. Cir. 1990); *Diversitech Corp. v. Century Steps, Inc.,* 850 F.2d 675, 7 USPQ2d 1315 (Fed. Cir. 1988); *Constant v. Advanced Micro-Devices, Inc.,* 848 F.2d 1560, 7 USPQ2d 1057 (Fed. Cir. 1988); *Alco Standard Corp. v. TVA,* 808 F.2d 1490, 1 USPQ2d 1337 (Fed. Cir. 1986); *In re Marshall,* 578 F.2d 301, 198 USPQ 344 (CCPA 1978); *In re Arkley,* 455 F.2d 586, 172 USPQ 524 (CCPA 1972). Moreover, it is incumbent upon the examiner to identify wherein each and every facet of the claimed invention is disclosed in the applied reference. *Lindemann Maschinenfabrik GmbH v. American Hoist and Derrick,* 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984).

Each of the independent claims on appeal defines a polymeric balloon which is "biaxially oriented." Ergo, in order to establish a *prima facie* basis to defeat the patentability of independent claims 13 and 25 under 35 U.S.C. §102, the examiner is obliged to point out where Schjeldahl discloses a *biaxially oriented* polymeric balloon. The tenor of the final rejection and Answer presupposes that Schjeldahl discloses a biaxially oriented polymeric balloon. See, for example, page 5 of the Final Rejection wherein the examiner states

> [t]he reference clearly teaches a biaxially oriented balloon catheter, and states that it is made by injection blow molding.

See, also, page 5 of the Answer wherein the examiner states

> [a]rguments that the references don't disclose a biaxially oriented PET (polyethylene terephthalate) balloon catheter is contrary to what is *clearly stated* in the references (emphasis supplied).

The examiner does not point to, and we do not find, any express disclosure in Schjeldahl of a biaxially oriented polymeric balloon.

It would appear that the relevant evulgations in Schjeldahl which may have led the examiner to his determination are:

(a) an expander [3] formed *from* a thin, flexible inelastic, high tensile strength, *biaxially oriented* synthetic plastic material (column 2 of Schjeldahl '989, lines 63 through 65, emphasis supplied);

(b) The expander 30 is preferably formed *from* a suitable synthetic plastic material, such as *biaxially oriented* polypropylene, *by an injection blow molding operation* and, as such, is substantially inelastic in both the axial and radial directions and may, for example, have a finished wall thickness in the range of from 0.005 to 0.200 millimeters, 0.025 millimeters being typical (column 6 of Schjeldahl '989, lines 45 through 52, emphasis supplied);

(c) It has been found that an expander of the above-dimensional characteristics can withstand internal inflation pressure in excess of 7 atmospheres without fear of rupture (column 6 of Schjeldahl '989, lines 62 through 65);

(d) injection blow molding step used to form the expander 30 (column 8, lines 16 and 17);

(e) the expander 30 is formed *from* a *biaxially oriented* thin plastic material capable of withstanding relatively high internal pressures without rupture and without exceeding the elastic limit for the material itself (column 10 of Schjeldahl '989, lines 32 through 36, emphasis supplied);

(f) the expander 82 is preferably formed *from* a suitable synthetic plastic material such as *biaxially oriented polypropylene* or *biaxially oriented polyethylene terephthalate by an injection molding operation* and, as such, is substantially inelastic in both the axial and radial direction (column 12 of Schjeldahl '989, lines 22 through 37, emphasis supplied); and

(g) Apparatus as in claim 1 wherein said non-elastic expander member comprises a longitudinally extending thin, flexible, tubular element *formed from a biaxially oriented* synthetic plastic material surrounding said outer tubular member with opposed ends thereof secured to said outer tubular member at spaced apart locations proximate said distal end thereof (claim 8 of Schjeldahl '989, emphasis supplied).

These excerpts do not justify the determination that Schjeldahl discloses a biaxially oriented polymeric balloon.

According to Schjeldahl, the *starting* material is a biaxially oriented synthetic plastic material, such as polyethylene terephthalate. The *final article, i.e.,* the expander or catheter balloon, is *not characterized as biaxially oriented.* Moreover, it would appear to be *undisputed* that the *only* method disclosed by Schjeldahl for transforming the biaxially oriented *starting* plastic into the *final* catheter balloon, *i.e.,* injection blow molding, is *not* capable of producing a biaxially oriented catheter balloon. In fact, it is *undisputed* that injection blow molding would *destroy* the biaxial orientation of the plastic starting material. We refer to the Belcher affidavits, Exhibits V, VI and VIII,[4] which factually set forth the differences between "injection blow molding" and "injection stretch blow molding," and support the conclusion that the "injection blow molding" process disclosed by Schjeldahl could not possibly produce a biaxially oriented polymeric balloon.[5]

Indeed, the examiner agrees with appellant's position that injection blow molding could *not* produce a biaxially oriented balloon. See, for example, page 5 of the Final Rejection wherein the examiner states:

> [s]tatements that injection blow molding without stretching will not produce a biaxially oriented article are *true* . . . (emphasis supplied).

The examiner goes on, in the same sentence, to state:

> but since the reference produces a biaxially oriented article, clearly a stretching step must be used.

Again, on page 5 of the Answer, the examiner states:

> Since Schjeldahl et al produces a biaxially oriented article it follows that a stretching step must be used in the injection blow molding process.

The inescapable facts are that Schjeldahl does not disclose a biaxially oriented catheter balloon and does not mention a stretching step.

[2] The examiner also relies upon the theory that Schjeldahl's catheter balloon is inherently biaxially oriented. On page 4 of the Answer, the examiner points out that inasmuch as the Patent and Trademark Office does not have the requisite laboratory equipment for testing, the burden shifts to appellant. However, the initial burden of establishing a *prima facie* basis to deny patentability to a claimed invention rests

[3] Schjeldahl characterizes the catheter balloon as an expander.

[4] Unless otherwise indicated, all exhibits mentioned are the exhibits to appellant's Brief.

[5] We recognize that a high burden of proof is required to demonstrate the inoperability of a United States patent. *In re Weber,* 405 F.2d 1403, 160 USPQ 549 (CCPA 1969); *In re Michalek,* 162 F.2d 229, 74 USPQ 107 (CCPA 1947). However, as noted above, Schjeldahl does not disclose a catheter balloon made *of* a biaxially oriented plastic. Therefore, appellant's evidence is not an attack on the operability of Schjeldahl, but quite relevant to the issue of inherency, *i.e.,* whether the catheter balloon disclosed by Schjedahl is inherently biaxially oriented.

upon the examiner. *In re Piasecki*, 745 F.2d 1468, 223 USPQ 785 (Fed. Cir. 1984). In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic *necessarily* flows from the teachings of the applied prior art. *In re King*, 801 F.2d 1324, 231 USPQ 136 (Fed. Cir. 1986); *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983); *In re Oelrich*, 666 F.2d 578, 212 USPQ 323 (CCPA 1981); *In re Wilding*, 535 F.2d 631, 190 USPQ 59 (CCPA 1976); *Hansgirg v. Kemmer*, 102 F.2d 212, 40 USPQ 665 (CCPA 1939). In our opinion, the examiner has not discharged that initial burden.

Schjeldahl does not provide any working example revealing the process conditions employed to produce the catheter balloon. We have *only* a general invitation to employ "injection blow molding." As previously discussed, it is undisputed that injection blow molding would *not* have produced a biaxially oriented balloon and would have destroyed the biaxially orientation of a polymeric starting material.

Schjeldahl does not disclose any particular tensile strength of the catheter balloon. We do not find sufficient factual basis or cogent scientific reasoning to support the conclusion that Schjeldahl's disclosure with respect to the ability of the catheter balloon to "withstand an internal inflation pressure in excess of 7 atmospheres without fear of rupture" (column 6 of Schjeldahl '989, lines 63 through 65) *necessarily* means that the catheter balloon is biaxially oriented. According to the membrane equation calculations reported in Levy's declaration (Exhibit IV), Schjeldahl's balloon could not possibly exhibit the tensile characteristics of a biaxially oriented balloon. Levy's calculations are *inconsistent* with those of Pinchuk (Exhibit III). Suffice it to say, the conflicting calculations taint the factual determination of inherency with impermissible conjecture. Indeed, the examiner, in the paragraph bridging pages 4 and 5 of the Answer, states that

> the membrane equation used to determine the tensil [sic, tensile] strength can be manipulated to produce any desired value, and thus is misleading.

Nevertheless, the examiner goes on to favor Pinchuk's calculations by stating in that same paragraph that

> [c]ertainly use of the typically used wall thickness disclosed in Schjeldahl et al with the average radius, as done in the Pinchuk Declaration would be reasonable.

As noted above, the conflicting results obtained by applying the membrance equation, and the examiner's acknowledgment that that equation "can be manipulated to produce any desired value," underscore the speculative nature upon which the determination of inherency rests.

We do not find sufficient cogent technical reasoning and/or objective evidence to support the conclusion that Schjeldahl's characterization of the catheter balloon as inelastic in the axial and radial direction *necessarily* means that the catheter balloon is biaxially oriented. The characteristic "inelastic," as employed by Schjeldahl, apparently means that the catheter balloon will expand to a preformed diameter to enable precise measurement of the pressures exerted on the inner wall of the artery during the dilation procedure (column 4 of Schjeldahl '989, lines 12 through 17).

[3] In summary, Schjeldahl does not disclose a biaxially oriented catheter balloon. We do not find a sufficient basis to support the determination that Schjeldahl's balloon is *inherently* (necessarily) biaxially oriented. *In re King, supra; W.L. Gore & Associates, Inc. v. Garlock, Inc., supra; In re Oelrich, supra; In re Wilding, supra; Hansgirg v. Kemmer, supra.* Accordingly, the examiner's rejection of claims 13, 14, 16, 17 and 25, under 35 U.S.C. §102 as anticipated by Schjeldahl is reversed.[6]

The Rejection of Claims 13 through 17 under 35 U.S.C. §103 Based upon the Combined Disclosures of Schjeldahl and Wyeth.

Wyeth is directed to producing high strength biaxially oriented polyethylene terephthalate beverage containers. The disclosed method involves stretching polyethylene terephthalate having a relatively high inherent viscosity; *e.g.*, at least about 0.85.

[6] There is evidence of record that Dupont, the assignee of the application, furnished biaxially oriented polyethylene terephthalate to Schjeldahl when he informed Dupont personnel that he required a thin, high strength polymeric film having a tensile strength in the range of 20,000–40,000 psi. See the Schjeldahl affidavit (Exhibit VIII) and the Dengler declaration executed on May 21, 1988 and appended to the protest submitted in parent application Serial No. 914,108. Such facts are not inconsistent with our determination that Schjeldahl does not disclose a biaxially oriented polyethylene terephthalate catheter balloon. The Rydell affidavit appended to the protest in the parent application does not persuade us that Schjeldahl expressly or inherently discloses a biaxially oriented polymeric catheter balloon. See Belcher's affidavit (Exhibit VI).

It is apparent from the Final Rejection and Answer that the examiner's rejection of the appealed claims under 35 U.S.C. 103 is *not* predicated upon the theory that one having ordinary skill in the art would have been led to employ Wyeth's technique to produce a biaxially oriented balloon for use in Schjeldahl's catheter. Instead, the examiner presupposes that Schjeldahl discloses a biaxially oriented catheter balloon. The examiner relies upon Wyeth *solely* for the disclosed use of high viscosity polyethylene terephthalate tubing. We refer to page 6 of the Answer, first complete paragraph, wherein the examiner explains the rejection by stating:

> Wyeth et al is not being combined with Schjeldahl et al, but merely shows the claimed high viscosity PET (polyethylene terephthalate) and supports the examiners [sic, examiner's] inherency arguments.' ... The examiner is not substituting the process of Wyeth et al into Schjeldahl et al since both disclose the same process.[8] Arguments that Wyeth et al can't be scaled down are irrelevant since the examiner is not seeking to scale down that reference to produce the claimed article.

[4] We have already concluded that the examiner factually erred in determining that Schjeldahl expressly or inherently discloses a biaxially oriented catheter balloon. Assuming, *arguendo*, the examiner correctly concluded that one having ordinary skill in the art would have been led to employ a high viscosity polyethylene terephthalate tubing in producing Schjeldahl's catheter balloon, the rejection under 35 U.S.C. §103 must fall because the examiner has not established that the resulting catheter balloon is biaxially oriented. *Uniroyal, Inc. v. Rudkin-Wiley Corp.*, 837 F.2d 1044, 5 USPQ2d 1434 (Fed. Cir. 1988).

Inasmuch as the examiner's rejection under 35 U.S.C. §103 is not predicated upon the theory that one having ordinary skill in the art would have been led to employ a conventional stretch blow molding technique, such as that disclosed by Wyeth, to produce Schjeldahl's catheter balloon, the motivation for such a combination is an issue which was not crystallized on appeal and was not confronted by appellant. However, in view of the examiner's gratuitous statement in the paragraph bridging pages 5 and 6 of the Answer,[9] we are constrained to address that issue.

There appears to be no dispute that one having ordinary skill in the art would have recognized the desirability of producing a biaxially oriented balloon for use in Schjeldahl's catheter, since biaxially oriented materials were known to exhibit high tensile strengths. The thrust of the evidence relied upon by the examiner is that one having ordinary skill in the art would have simply resorted to a conventional stretch molding technique to produce a biaxially oriented balloon for use in Schjeldahl's catheter, specifically, *the technique employed by Wyeth to produce a beverage container*. See paragraph 4 of the Rydell affidavit executed April 25, 1988 and offered in support of the protest in parent application Serial No. 914,108, paragraph 5 of the Pinchuk affidavit (Exhibit III), and paragraphs 4 and 5 of the Kaufman affidavit (Exhibit XII). Interestingly enough, *Wyeth disagrees*. See page 5 of Wyeth's declaration (Exhibit XI). Wyeth points out various differences between the PET bottles produced by his disclosed process and the requirements of a catheter balloon, and then concludes that his process could *not* be used to produce a catheter balloon of the type disclosed by Levy.

We are persuaded by Belcher's affidavits and Wyeth's declaration, notwithstanding the affidavits of Rydell, Pinchuk and Kaufman,[10] that the known processes for produc-

[7] Actually, according to the Final Rejection which is incorporated in the Answer,

> [i]t is the Examiner's position that it would be *prima facie* obvious to use the high viscosity polyethylene terephthalate of Wyeth in Schjeldahl et al to produce the claimed product (page 4, the only complete paragraph).

[8] It is apparent from our reversal of the examiner's rejection under 35 U.S.C. §102 that, in our opinion, Schjeldahl discloses neither a biaxially oriented catheter balloon nor a molding process which involves stretching.

[9] The noted statement provides:

> Certainly in the least there was an *invitation* to make a biaxially oriented catheter balloon at the time of the Schjeldahl et al invention. Additionally injection stretch blow molding to produce biaxially oriented articles was well known at the time of the Schjeldahl et al invention (emphasis supplied).

[10] We agree with appellant that the credentials of Belcher and Wyeth in the relevant art appear more impressive than those of protestor's experts. According to the affidavit appearing as Appendix V, Belcher authored the chapter called "Blow Molding of Polymers" for the fifth edition of the Plastic Engineering Handbook of the Society of Plastics Industry. In addition, Belcher authored two chapters, one on "injection blow molding" and one on "stretch blow molding" for the Blow Molding Handbook of the Society of Plastics and Engineers. We consider Wyeth's opinion with respect to the capabilities of his own invention entitled to greater weight than the opinions of Rydell, Pinchuk and Kaufman.

ing biaxially oriented beverage containers, such as that disclosed by Wyeth, could not have been simply scaled down to produce a biaxially oriented catheter balloon for use in medical dilation procedures without the exercise of inventive skill.[11] Based upon the record before us, it would appear unrealistic to conclude that one having ordinary skill in the art would have been led to employ Wyeth's technique, which is designed to produce beverage containers, to produce Schjeldahl's catheter balloon, motivated by a *reasonable expectation* of obtaining a *biaxially oriented* polymeric catheter balloon. *In re O'Farrell*, 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988). The rejection under 35 U.S.C. §103 is also reversed.

REVERSED.

U.S. Patent and Trademark Office
Trademark Trial and Appeal Board

The Ritz Hotel Limited v. Ritz Closet Seat Corp.

Opposition No. 78,707

Decided September 24, 1990

TRADEMARKS AND UNFAIR TRADE PRACTICES

1. Practice and procedure in Patent and Trademark Office — Interpartes proceedings — Standing (§325.0303)

Practice and procedure in Patent and Trademark Office — Interpartes proceedings — Opposition and cancellation — Rules and rules practice (§325.0305.05)

Opposer may, on rebuttal, introduce facts and witnesses appropriate to deny, explain, or otherwise discredit applicant's facts and witnesses, but testimony of opposer's witnesses that was directed toward applicant's testimony regarding damage should have been introduced, if at all, as part of opposer's case-in-chief, since damage issue has relevance only to opposer's standing to be heard; since applicant's testimony has not challenged opposer's standing, testimony given by opposer's witnesses during rebuttal period is improper.

2. Acquisition, assignment, and maintenance of marks — Scope of trademark — Expansion of goods/territory (§305.0206)

Trademark owner possesses rights in mark sufficient to preclude subsequent user's registration of same or substantially similar mark not only for like or similar goods, but for any goods which might reasonably be expected to emanate from it in normal expansion or extension of its business; personal luxury items are clearly within natural scope of expansion of opposer's business, which is providing hotel services under mark "Ritz," but toilet seats are not within such natural expansion of business.

3. Infringement; conflicts between marks — Likelihood of confusion — Relatedness of goods or services — Not similar (§335.0305.05)

Applicant's toilet seats, sold under mark "Rit-Z," are not likely to be attributed to opposer, which provides hotel services and sells luxury items under mark "Ritz."

4. Registration and its effects — Non-registrable subject matter — Immoral, deceptive, scandalous (§315.0403)

Opposer, in order to succeed on claim under Trademark Act's Section 2(a), must demonstrate that applicant's mark is same as, or close approximation of, opposer's name or identity, that applicant's mark would be recognized as such, that opposer is not connected with applicant's activities under its mark, and that opposer's name or identity is of sufficient fame or reputation that when applicant's mark is used on its goods, connection to opposer would be presumed; opposer which has failed to show connection of applicant's mark "Rit-Z," for toilet seats, with its hotel services and goods under mark "Ritz" has failed to prove that applicant's use of its mark points uniquely to opposer and thus has failed to set forth claim under Section 2(a).

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[11] We find it somewhat unrealistic in light of the apparent disparities in size and function, Belcher's affidavits and Wyeth's declaration, that Pinchuk and Kaufman equate beverage bottles to catheter balloons. See paragraph 10 of the Pinchuk affidavit (Exhibit III), wherein it is stated

> [a]s a blow molded polymeric article, a bottle and a catheter balloon are equivalent.

See, also, paragraph 4 of the Kaufman affidavit (Exhibit XII), wherein it is stated that

> anyone with ordinary skill in the plastics art would know how to make a biaxially oriented PET balloon; it would be similar to making a biaxially oriented PET bottle because both catheter balloons and bottles are equivalent structures — they are both fluid containers.

Opposition filed by The Ritz Hotel Limited to application, serial no. 679,883, filed Aug. 21, 1987, by Ritz Closet Seat Corp. Opposition dismissed.

Pennie & Edmonds, New York, N.Y., for The Ritz Hotel Limited.

Zarley, McKee, Thomte, Voorhees & Sease, Des Moines, Iowa, for Ritz Closet Seat Corp.

Before Rooney, Seeherman, and Hohein, members.

Rooney, member.

An opposition has been filed against registration of the mark RIT-Z, in the form shown below, for toilet seats

Use since February 1987 was alleged. The grounds for opposition are, essentially, that the Ritz Hotel is now and for many years has been considered to be one of the finest hotels in the world; that in conjunction with its hotel business the Ritz Hotel distributes and sells a wide variety of products bearing its name RITZ; that since long prior to the alleged date of applicant's first use in commerce of the designation RIT-Z, the Ritz Hotel has been, and is now, conducting its business under the trade name, trading style and corporate identification The Ritz Hotel Limited; that the Ritz Hotel is now and, since long prior to February 1987 has been known and referred to among the trade and the public as the "RITZ"; that applicant knew or should have known that the designation RIT-Z was derived from or refers to the Ritz Hotel, or that the public is likely to believe that RIT-Z refers to the Ritz Hotel; that applicant's mark, RIT-Z, so resembles the following marks previously used and duly registered by opposer, the Ritz Hotel Limited, as to be likely to cause confusion, mistake or deception:

HOTEL RITZ PARIS and design for bath mats.[1]

HOTEL RITZ PARIS for bath mats.[2]

RITZ PARIS RITZ HOTEL and design for furniture, mirrors, picture frames, wastepaper baskets, and decorative objects made of wood, cork, reed, cane wicker, horn, bone, ivory, whalebone, shell, amber, mother-of-pearl, meerschaum and substitutes including plastic, objects of art, namely, bric-a-brac, statuettes and boxes.[3]

RITZ PARIS RITZ HOTEL and design for electrical household appliances for domestic use, namely, food warmers, plate warmers, grills, food cookers and coffee makers and electric stoves and furnaces,[4] and

RITZ for electrical household appliances for domestic use, namely, food warmers, plate warmers, grills, food cookers and coffee makers and electric stoves and furnaces.[5]

As a second ground for opposition, opposer alleges that the designation, RIT-Z, consists of and comprises deceptive matter which may falsely suggest a connection with the Ritz Hotel in violation of Section 2(a) of the Trademark Act; that The Ritz Hotel will be damaged by the registration which applicant seeks because such registration will support and assist applicant in the confusing and misleading use of applicant's designation RIT-Z for toilet seats to the derogation of opposer's prior rights.

Applicant denied the significant allegations of the notice of opposition.

The record contains status and title copies of opposer's registrations introduced during the testimony of opposer's witness, a number of third-party registrations submitted by applicant under a notice of reliance and testimony with exhibits on behalf of each party. Both parties filed briefs. Neither party requested an oral hearing.

Opposer is the owner of the Ritz Hotel in Paris. The Ritz Hotel was founded by Cesar Ritz in 1898. Cesar Ritz introduced a number of innovations into the hotel business. It was he who first decided that every room should have its own bathroom, that rooms should have lighted closets, that there should be 24-hour room service and that the sheets should be changed daily rather than every other day, as was the practice at that time. Mr. Ritz also introduced oversized bathtubs in his hotel. (Klein deposition, p.6) The high

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[1] Registration. No. 1, 375, 808 issued December 17, 1985

[2] Registration. No. 1, 355, 328 issued August 20, 1985

[3] Registration. No. 1, 380, 466 issued January 28, 1986

[4] Registration. No. 1, 390, 678 issued April 22, 1986

[5] Registration. No. 1, 448, 340 issued July 21, 1987

(1) The Secretary's Motion to Dismiss for Lack of Jurisdiction is granted.

(2) Each party shall bear its own costs.

In re Albert M.A. RIJCKAERT and Joannes A.E. Van Der Kop.

No. 93–1206.

United States Court of Appeals, Federal Circuit.

Nov. 23, 1993.

Appeal was taken from a decision of the Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences affirming final rejection of claims of patent application relating to apparatus for recording and reproducing electric signal on magnetic record carrier. The Court of Appeals, Lourie, Circuit Judge, held that application was not unpatentable on ground of obviousness.

Reversed.

1. Patents ⇐324.5

Court of Appeals reviews de novo Board of Patent Appeals and Interferences' ultimate determination of obviousness. 35 U.S.C.A. § 103.

2. Patents ⇐324.55(2)

Underlying factual inquiries, such as scope and content of prior art, differences between prior art and claimed invention, and level of ordinary skill in the art are reviewed for clear error in patent infringement action.

3. Patents ⇐32

In rejecting claims on ground of obviousness, examiner bears initial burden of presenting prima facie case of obviousness; only if that burden is met, does burden of coming forward with evidence or argument shift to applicant. 35 U.S.C.A. § 103.

4. Patents ⇐36(1)

If examiner fails to establish prima facie case of obviousness, rejection of patent claim is improper and will be overturned. 35 U.S.C.A. § 103.

5. Patents ⇐36(3)

Patent examiner failed to establish prima facie case that application relating to apparatus for recording and reproducing electric signal on magnetic record carrier was unpatentable as being obvious; primary reference disclosing signal processing circuit for video recording and reproducing apparatus was primarily concerned with processing high quality broadcast television signal and did not describe use of time expansion and compression as a means of optimally filling tracks and secondary reference did not remedy deficiencies of primary reference. 35 U.S.C.A. § 103.

Edward W. Goodman, North American Philips Corp., of Tarrytown, NY, argued for appellant. With him on the brief was Algy Tamoshunas.

Lee E. Barrett, Associate Sol., Office of the Sol., Arlington, VA, argued for appellee. With him on the brief was Fred E. McKelvey, Sol.

Before MAYER and LOURIE, Circuit Judges, and LAY*, Senior Circuit Judge.

LOURIE, Circuit Judge.

Albert Rijckaert and Joannes van der Kop ("Rijckaert") appeal from the decision of the United States Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences affirming the final rejection of claims 5–12, all of the pending claims in patent application serial no. 07/345,396, as being unpatentable under 35 U.S.C. § 103 (1988). Because the references relied upon to reject

* Honorable Donald P. Lay, Senior Circuit Judge, United States Court of Appeals for the Eighth Circuit, sitting by designation.

the claims do not provide the basis for a *prima facie* determination that the claimed invention would have been obvious, we reverse.

BACKGROUND

The patent application at issue relates to an apparatus for recording and reproducing an electric signal on a magnetic record carrier. Independent claim 11 is drawn to a recording apparatus and it specifies a relationship between time expansion or compression and three variables, α, n, and M. Claim 11 reads, in pertinent part:

> 11. An apparatus for recording an electric signal on a magnetic record carrier in tracks which are inclined relative to the longitudinal direction of said record carrier, comprising: ...
>
> ....
>
> ... [a] time-base correction circuit provid[ing] a time expansion or time compression of the signal blocks by a factor of $\alpha^*n/(180^*(M+1))$, where α is the wrapping angle of the record carrier around the head drum and differs from 180°, n is the number of head pairs, and M is the number of times within a specific time interval that a head pair which comes in contact with the record carrier during said time interval does not record a signal on the record carrier, said time interval being defined by those instants at which two consecutive track pairs are recorded by one or two head pairs.

Independent claim 12 is drawn to an apparatus for reproducing a recorded signal and it recites the reciprocal relationship between time compression or expansion and the three variables α, and M. Dependent claims 5–10 further limit claims 11 or 12.

The Board upheld the final rejection of claims 5 and 7–12 under 35 U.S.C. § 103 as being unpatentable over U.S. Patent 4,757,392 to Awamoto in view of Driessen et al., *An Experimental Digital Video Recording System*, CE–32 I.E.E.E. Transactions on Consumer Electronics 3, Aug. 1986, at 362–70. The Board also upheld the final rejection of claim 6 as being unpatentable over Awamoto and Driessen in view of U.S. Patent 4,542,417 to Ohta.

DISCUSSION

[1, 2] We review *de novo* the Board's ultimate determination of obviousness. *In re De Blauwe*, 736 F.2d 699, 703, 222 USPQ 191, 195 (Fed.Cir.1984). Underlying factual inquiries, such as the scope and content of the prior art, differences between the prior art and the claimed invention, and level of ordinary skill in the art are reviewed for clear error. *See In re Caveney*, 761 F.2d 671, 674, 226 USPQ 1, 3 (Fed.Cir.1985).

[3, 4] In rejecting claims under 35 U.S.C. § 103, the examiner bears the initial burden of presenting a *prima facie* case of obviousness. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed.Cir.1992). Only if that burden is met, does the burden of coming forward with evidence or argument shift to the applicant. *Id.* "A *prima facie* case of obviousness is established when the teachings from the prior art itself would appear to have suggested the claimed subject matter to a person of ordinary skill in the art." *In re Bell*, 991 F.2d 781, 782, 26 USPQ2d 1529, 1531 (Fed.Cir.1993) (quoting *In re Rinehart*, 531 F.2d 1048, 1051, 189 USPQ 143, 147 (CCPA 1976)). If the examiner fails to establish a *prima facie* case, the rejection is improper and will be overturned. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed.Cir.1988).

[5] All of the claims except claim 6 stand rejected under 35 U.S.C. § 103 as being obvious over Awamoto in view of Driessen.[1] Awamoto, the primary reference, discloses a signal processing circuit for a video recording and reproducing apparatus. Awamoto specifically discloses the time expansion of an input signal by a factor of two and the corresponding time compression of an output signal in a manner inverse to that of the time expansion. Further, Awamoto uses two video heads mounted on a rotary drum "of any

1. The claims stand or fall together since no separate argument for patentability has been made for each claim.

of a well known video tape loading mechanism such that [the heads] follow parallel tracks skewed relative to the length of video tape." Driessen discloses a recording system using two pairs of heads mounted on piezo-ceramic actuators.

The Board concluded that the subject matter of the claims would have been obvious over Awamoto in view of Driessen, stating that "the time expansion or time compression relationship is satisfied for the expansion of two disclosed [in] Awamoto when a wrapping angle of 360°, one pair of heads and no non-recording intervals are assumed." The Board further asserted that the recognition of the claimed relationship between time expansion/compression and the three variables *a*, n, and M is "the mere discovery of a relationship that is applicable to [a] prior art apparatus[, and] does not [give] rise to a patentable invention." Thus, in affirming the rejection, the Board first assumed that the claim limitation at issue, the relationship between time expansion/compression and the three variables, was somehow "inherent" in the prior art as shown by Awamoto. The Board also assumed specific values for the claimed variables in order to assert that Awamoto's device satisfies the claimed relationship.

Rijckaert argues that the examiner has not established a *prima facie* case of obviousness and that the examiner's assumptions do not constitute the disclosure of prior art. We agree. Awamoto does not disclose the wrapping angle of the record carrier around the head drum or the number of times that a head pair which comes in contact with the record carrier does not record a signal on the record carrier. Nor does Awamoto discuss the claimed relationship of the three variables to time expansion/compression.[2] Driessen, the secondary reference, is relied upon only to teach the provision of a pair of write heads having a mechanically rigid coupling to each other and does not remedy the deficiencies of Awamoto. Thus, the prior art relied upon does not disclose, suggest, or render obvious the claimed invention, either individually or when combined.[3]

Awamoto does not describe the use of time expansion and compression as a means of optimally filling tracks, much less suggest that the three variables of the claims are even a factor in determining the amount of time expansion or time compression. Rather, Awamoto is concerned primarily with processing a high-quality broadcast television signal for use in conventional video machinery, and with compensating for errors introduced to such a signal by a transfer circuit. The Commissioner's assertion "that the [analysis discussed in his brief] and Awamoto demonstrate that the relationship was, in fact, well known in the art" is unavailing. While the court appreciates the Commissioner's thorough explanation of the claimed relationship in his brief, the Commissioner's brief is not prior art. The prior art is Awamoto, and it does not indicate that the relationship is well known in the art, nor does it suggest the claimed relationship. *See In re Yates*, 663 F.2d 1054, 211 USPQ 1149, 1151 (CCPA 1981) (when the PTO asserts that there is an explicit or implicit teaching or suggestion in the prior art, it must indicate where such a teaching or suggestion appears in the reference).

To support the Board's affirmance of the rejection, the Commissioner points out that in the recording art, the exact matching of signal time to recording time is an optimal

2. The Commissioner admits that other limitations recited in claims 11 and 12 are not found in Awamoto; however, those limitations were not argued before the Board or this court. Thus, we agree with the Commissioner that those limitations are not at issue here.

3. The Board also noted that the claims are not "specific" in that they claim the three variables as a "factor" of the expansion or compression time. The Board stated, "claims 11 and 12 fail to say which of expansion time or compression time is factored by the variables, how or when one of the two times is selected based on the variables or how each of the two times is related to the variables." The Board further stated, "the relationship is probably satisfied by any prior art video tape recording and reproducing apparatus that otherwise satisfies the remaining requirements of the claims at bar." While the Board's position implies a possible rejection based upon 35 U.S.C. § 112, this issue is not before us. In any event, the statement that the relationship is "probably satisfied" by the prior art is speculative and therefore does not establish a *prima facie* case of unpatentability.

condition, and that this condition would be met by fulfilling the claimed relationship. While the condition described may be an optimal one, it is not "inherent" in Awamoto. Nor are the means to achieve this optimal condition disclosed by Awamoto, explicitly or implicitly. "The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient [to establish inherency.]" *In re Oelrich*, 666 F.2d 578, 581–82, 212 USPQ 323, 326 (CCPA 1981) (citations omitted) (emphasis added). "That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown." *In re Spormann*, 363 F.2d 444, 448, 150 USPQ 449, 452 (CCPA 1966). Such a retrospective view of inherency is not a substitute for some teaching or suggestion supporting an obviousness rejection. *See In re Newell*, 891 F.2d 899, 901, 13 USPQ2d 1248, 1250 (Fed.Cir.1989).

Rijckaert also argues that the rejection of dependent claim 6 as being obvious over Awamoto and Driessen in view of Ohta is improper. Ohta discloses an apparatus for compensating for signal loss in a single-head video recorder using a time compression factor of 3/5 (a signal of time period 5t/4 is compressed into a track of time period 3t/4) so that a signal is recorded completely during the time period that it takes the recording head to scan the magnetic tape. Regarding the Ohta patent, the examiner stated, "Ohta was only relied upon to support the idea that other compression factors are used in the prior art...."[4] The relationship between the time expansion/compression and the three variables recited in the claims from which claim 6 depends, which is absent in the combination of Awamoto and Driessen, is not supplied by Ohta. Thus, we agree that the rejection of claim 6 under § 103 is improper for the reasons set forth above with respect to the other claims.

While the Commissioner criticizes Rijckaert's arguments regarding the § 103 rejections, the burden to rebut a rejection of obviousness does not arise until a *prima facie* case has been established. In the case before us, it was not.

CONCLUSION

The decision of the United States Patent and Trademark Office Board of Patent Appeals and Interferences affirming the final rejection is reversed.

REVERSED.

4. The Board did not specifically address the rejection of claim 6; therefore, claim 6 was considered to be affirmed for the reasons stated by the examiner. *See* 37 C.F.R. § 1.196(a) (1993).

Application of Donald Francis BEST, Anthony Peter Bolton and Herbert Charles Shaw.

Patent Appeal No. 77-509.

United States Court of Customs and Patent Appeals.

Oct. 13, 1977.

Applicant appealed from a decision of the Patent and Trademark Office Board of Appeals, Serial No. 347,216, sustaining rejections of claims on an application for "Catalyst for Hydrocarbon Conversion Processes and Process for Preparing Same." The Court of Customs and Patent Appeals, Markey, Chief Judge, held that product and process claims were properly rejected as anticipated or as obvious in light of prior art.

Affirmed.

1. Patents ⇐18, 66(1.12, 1.24)

Product and process claims of application for "Catalyst for Hydrocarbon Conversion Processes and Process for Preparing Same," were properly rejected as anticipated or as obvious in light of prior art. 35 U.S.C.A. §§ 102, 103.

2. Patents ⇐66(1.12)

Indirect comparisons, based on established scientific principles, can validly be applied to distinguish claimed chemical process of product from that disclosed in prior art. 35 U.S.C.A. § 103.

3. Patents ⇐32, 58

Where claimed and prior art products are identically or substantially identical, or are produced by identical or substantially identical processes, Patent and Trademark Office can require applicant to prove that prior art products do not necessarily or inherently possess characteristics of his claimed product; and whether rejection is based on inherency or on prima facie obviousness, jointly or alternatively, burden of proof is the same and its fairness is evidenced by Patent and Trademark Office's inability to manufacture products or to obtain and compare prior art products. 35 U.S.C.A. §§ 102, 103.

4. Patents ⇐18, 66(1)

There is nothing inconsistent in concurrent rejection of patent claims for obviousness and for anticipation by inherency. 35 U.S.C.A. §§ 102, 103.

Richard G. Miller, New York City, attorney of record, for appellants, James C. Arvantes, Arlington, Va., of counsel.

Joseph F. Nakamura, Washington, D. C., for the Commissioner of Patents, Gerald H. Bjorge, Washington, D. C., of counsel.

Before MARKEY, C. J., RICH, BALDWIN and LANE, JJ., and FORD, J., United States Customs Court.

MARKEY, Chief Judge.

Appeal from the decision of the Patent and Trademark Office (PTO) Board of Appeals (board) sustaining rejections of claims 1–7 under 35 U.S.C. § 102 or 35 U.S.C. § 103, and claims 3–7 under 35 U.S.C. § 112, of appellants' application serial No. 347,216, filed April 2, 1973, for "Catalyst for Hydrocarbon Conversion Processes and Process for Preparing Same."[1] We affirm.

The Invention

The invention relates to zeolitic molecular sieve catalyst compositions useful in hydrocarbon conversion and to a process for producing them. Claim 1 is illustrative of the product claims:

1. A crystalline zeolitic aluminosilicate having a SiO_2/Al_2O_3 molar ratio of from 4.6 to 5.4, a face centered cubic unit cell having an a_o of greater than 24.45 to 24.55A, an Na_2O/Al_2O_3 molar ratio of not greater than 0.25, an adsorptive capacity in the dehydrated state for oxygen of at least 26 weight per cent at 100 mm Hg oxygen pressure and %ao183°C., an ion

1. A continuation-in-part of serial No. 145,900, filed May 21, 1971.

exchange capacity of from 0.15 to 0.35 and having the essential X-ray powder diffraction pattern of zeolite Y with the proviso that the d-spacing thereof having the Miller Indices 331 is at least as great in intensity as the line thereof having the Miller Indices 533.

Claim 3 is illustrative of the process claims:

> 3. Process for preparing a hydrolytically-stable zeolitic aluminosilicate which comprises providing an ion-exchanged zeolite Y having the following composition in terms of mole ratios of oxides
>
> 0.75 %ao 0.9$(A)_2O$: 0.1 %ao 0.25 Na_2O: Al_2O_3: 4.6%ao5.4 SiO_2: yH_2O
>
> wherein "A" represents H^+ or NH_4^+ or a mixture thereof, and wherein y has a value of from zero to nine, heating the zeolite at a temperature between 550°C. and 800°C. for a period of at least 0.25 hours in an inert atmosphere comprising sufficient steam to prevent dehydroxylation of the zeolite, removing at least a major proportion of any ammonia generated by the heated zeolite from contact with the zeolite, and cooling the steamed zeolite to a temperature below 350°C. at a rate sufficiently rapid that the cooled zeolite exhibits an X-ray powder diffraction pattern having the d-spacing corresponding to the Miller Indices, hkl, of 331 at least as strong in intensity as that corresponding to the Miller Indices 533, prior to any post-steaming ion exchange treatment.

Claim 2 is restricted to a zeolite of claim 1 with a Na_2O/Al_2O_3 molar ratio of less than 0.038. Claims 4–7 add further process restrictions as to starting materials or process steps. All of the claims stand or fall with claims 1 and 3.

As recognized in the prior art, crystalline zeolitic aluminosilicates with high concentrations of sodium cations do not make good hydrocarbon conversion catalysts. For this reason sodium cations are replaced with non-metallic cations such as hydrogen or ammonium. The hydrogen or ammonium cations are removed by calcination, producing a decationized zeolite. Such decationized zeolites have poor hydrothermal stability, i. e., they lose their crystallinity upon reheating after contact with water.

The process of appealed claims 3–7 is a stabilization procedure for such low-sodium zeolites wherein a thermal treatment in the presence of steam is followed by a particular cool-down step. The zeolitic compositions of claims 1–2 represent the products of the claimed process.

The 102/103 Rejections

The references relied upon were:

Maher et al. (Maher)	3,293,192	Dec. 20, 1966
Hansford	3,354,077	Nov. 21, 1967
McDaniel et al. (McDaniel)	3,449,070	June 10, 1969
Kerr et al (Kerr I)	3,493,519	Feb. 3, 1970
Kerr (Kerr II)	3,513,108	May 19, 1970

All claims were rejected under 35 U.S.C. § 102 or 35 U.S.C. § 103 as unpatentable over Hansford. Claims 1–2 were additionally rejected in view of each of Maher, McDaniel, Kerr I, and Kerr II.[2]

Hansford discloses a method for producing a hydrothermally stable Y-sieve zeolite composition by calcining an ammonium zeolite Y for 2 or more hours in an atmosphere containing water vapor at a temperature of from 700°F to 1200°F (338°C–649°C). The starting material is disclosed by Hansford as having a SiO_2/Al_2O_3 molar ratio of 4 to 6 and a reduced Na_2O content of 0.6% to 2.5% by weight (appellants claim 0.1 %ao 0.25 Na_2O/Al_2O_3 molar ratio and disclose 2.48% by weight in example 10 of their specification). In rejecting claims 1–7 on Hansford, the examiner asserted that a major portion of any ammonia generated during calcination would inherently be removed from contact with the zeolite, because the gaseous atmosphere disclosed by Hansford was in the form of a moving stream. Also with respect to Hansford, the examiner believed the cooling rate of the zeolite after stabilization to be within the

2. The examiner rejected claims 1–7 under 35 U.S.C. § 103 as unpatentable over Kerr I, and claims 1, 2, 3, 6, and 7 under 35 U.S.C. § 103 as unpatentable over Kerr II. The board affirmed only in relation to claims 1–2 and reversed in relation to claims 3–7 over Kerr I and to claims 3, 6, and 7 over Kerr II.

terms of the appealed process claims. The claimed product being the unique result of the claimed process, the examiner, therefore, rejected both process and product claims as anticipated by Hansford, or, in any case, as obvious in view of Hansford.

In sustaining the rejection, the board added its view of Hansford.

> All the positive process limitations are expressly disclosed except for the functionally expressed rate of cooling. However, there is nothing to indicate that this rate of cooling in any way differs from the normal rate resulting from removal of the heat source. Thus, the examiner's conclusion that those parameters of the resultant product which are recited in the appealed claims but are not expressly disclosed in the reference would be inherent is a reasonable one, absent convincing evidence to the contrary. Appellants have presented no such convincing evidence. No comparison has been made between appellants' process and product and the process and product disclosed in the Hansford patent. The comparative data contained in appellants' specification and in an affidavit under 37 CFR 1.132 do not relate to the reference but merely illustrate the result of deviating from appellants' process. Such deviations appear to be also outside the scope of the Hansford teaching.

OPINION

I. The Process Claims

[1, 2] The appellants urge that, because Hansford is silent on appellants' crucial cool-down step and on his apparatus, a direct comparison between the claimed process and that of Hansford is impossible. Appellants correctly state that indirect comparisons, based on established scientific principles, can validly be applied to distinguish a claimed chemical process or product from that disclosed in the prior art. *In re Blondel*, 499 F.2d 1311, 182 USPQ 294 (CCPA 1974). However, our analysis of the comparative data offered by appellants convinces us that the burden of rebutting the PTO's reasonable assertion of inherency under 35 U.S.C. § 102, or of prima facie obviousness under 35 U.S.C. § 103, has not been met.

Our reading of Hansford leads us to conclude, as did the board, that all process limitations of claim 3 are expressly disclosed by Hansford, except for the functionally expressed rate of cooling. Because any sample of Hansford's calcined zeolitic catalyst would necessarily be cooled to facilitate subsequent handling, the conclusion of the examiner that such cooling is encompassed by the terms of the appealed claims was reasonable.

The board did not specifically mention the absence of ammonia as a result of "removing at least a major proportion of any ammonia generated by the heated zeolite from contact with the zeolite," as recited in claim 3. Its affirmance of the examiner, however, carried with it a concurrence in the examiner's view that Hansford discloses a gaseous atmosphere in a "stream." In concluding that Hansford expressly disclosed all process limitations except the cooling rate, the board necessarily considered Hansford's disclosure of a gas "stream" as equivalent to a disclosure of the removal of generated ammonia from contact with the zeolite. Though appellants argued before the board and before us that Hansford is silent on the matter, they have not provided any effective argument nor submitted any evidence that a gas stream does not inherently remove generated ammonia.

This court, in *In re Swinehart*, 439 F.2d 210, 58 CCPA 1027, 169 USPQ 226 (1971), set forth the burden of proof required to overcome an inherency rejection:

> [I]t is elementary that the mere recitation of a newly discovered function or property, inherently possessed by things in the prior art, does not cause a claim drawn to those things to distinguish over the prior art. Additionally, where the Patent Office has reason to believe that a functional limitation asserted to be critical for establishing novelty in the claimed subject matter may, in fact, be

an inherent characteristic of the prior art, it possesses the authority to require the applicant to prove that the subject matter shown to be in the prior art does not possess the characteristic relied on. [439 F.2d at 212–13, 58 CCPA at 1031, 169 USPQ at 229.]

This burden was involved in *In re Ludtke*, 441 F.2d 660, 58 CCPA 1159, 169 USPQ 563 (1971), and is applicable to product and process claims reasonably considered as possessing the allegedly inherent characteristics.

The proof required here relates to appellants' cool-down step. The only comparative data on the cool-down rate are found in examples 1(a) and 1(c) of appellants' specification. Those data merely establish that there may be cooling rates which are not the cooling rate functionally set forth in claim 3. Absent from the data is a comparison of X-ray diffraction patterns, the phenomenon employed in defining cooling rates. Thus the data found in the specification are insufficient to rebut the inherency rejection of the process claims.

In view of Hansford's silence on cool-down rate and on his apparatus, appellants need only have shown that the cool-down rate, for a typical laboratory-scale sample when employed in Hansford's process, would not yield a cooled zeolite with the X-ray diffraction pattern of claim 3. Appellants failed to do even that.

Appellants submitted an affidavit of Skeels,[3] the thrust of which was the assertion that, although cooling rates can vary greatly, depending on the apparatus employed and the quantity of zeolite treated, some normal cooling rates with typical laboratory equipment are much slower than that disclosed in appellants' specification and encompassed by claim 3. The Skeels affidavit fails for lack of a showing that such normal cooling rates are not rapid enough to result in the particular X-ray diffraction pattern recited in appealed claim 3.

We affirm the board's decision upholding the rejection of process claims 3–7 as anticipated under 35 U.S.C. § 102 or as obvious under 35 U.S.C. § 103, and do not reach the rejection of claims 3–7 under 35 U.S.C. § 112.

II. The Product Claims

Product claims 1–2 were rejected as unpatentable over each of Hansford, Maher, McDaniel, Kerr I, and Kerr II. We find it necessary to consider only Hansford.

[3, 4] Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. See *In re Ludtke, supra.* Whether the rejection is based on "inherency" under 35 U.S.C. § 102, on "prima facie obviousness" under 35 U.S.C. § 103, jointly or alternatively,[4] the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products. See *In re Brown*, 459 F.2d 531, 59 CCPA 1036, 173 USPQ 685 (1972).

In product claim 1 appellants have "fingerprinted" their crystalline zeolitic aluminosilicate by reciting six parameters, two directly compositional in nature, SiO_2/Al_2O_3 and Na_2O/Al_2O_3 molar ratios. The other parameters are the cubic unit cell size (a_o), the ion exchange capacity, the oxygen adsorption capacity, and the X-ray powder diffraction pattern. Hansford discloses SiO

3. The board considered the Skeels affidavit untimely and treated it as mere argument. But if the board's statement that appellants' cooling rate did not differ from "the normal rate resulting from removal of the heat source" were considered a new ground of rejection and the affidavit be considered evidence, the data presented would not rebut the inherency rejection, absent a showing of X-ray diffraction patterns for cooled zeolites.

4. There is nothing inconsistent in concurrent rejection for obviousness under 35 U.S.C. § 103 and for anticipation by inherency under 35 U.S.C. § 102. *In re Skoner*, 517 F.2d 947, 186 USPQ 80 (CCPA 1975); *In re Pearson*, 494 F.2d 1399, 181 USPQ 641 (CCPA 1974).

$_2/Al_2O_3$ and Na_2O/Al_2O_3 molar ratios within the ranges recited in claim 1, but does not specifically disclose the other parameters.

Though urging that the other parameters are the unique result of their claimed process, appellants have offered no comparison of those other parameters with the corresponding parameters of Hansford's product.

We affirm the decision of the board upholding the rejections of product claims 1–2 on Hansford and do not reach the rejections of claims 1–2 on Maker, McDaniel, Lerr I, of Kerr II.

The decision of the board is affirmed.

AFFIRMED.

Time has not shown any evidence of actual confusion. However, evidence of actual confusion is not necessary for establishing a claim of likelihood of confusion. *Miles Shoes, Inc. v. R.H. Macy & Co., Inc.*, 199 F.2d 602, 603 [95 USPQ 170, 171-72] (2d Cir. 1952), *cert. denied*, 345 U.S. 909 [96 USPQ 457] (1953); *La Touraine Coffee Co. v. Lorraine Coffee Co.*, 157 F.2d 115, 117 [70 USPQ 429, 431-32] (2d Cir. 1946), *cert. denied*, 329 U.S. 771 [71 USPQ 328] (1946). Moreover, in light of the circumstances of this injunction, this is not fatal to Time's claim of trade dress infringement. Globe has published only one issue of *Celebrity* which was on the stands for only a short period of time before Time brought this action. Given the prior findings of likelihood of confusion, it is understandable that Time would not have been able to gather such information as of the start of this action.

[4] For the reasons set forth above, Time has established a trade dress in the *People* cover format, the similarity of the *Celebrity* cover format, and that Globe's intentional imitation of *People*'s cover format in an effort to capitalize on their reputation and advertising. Further, the magazines are very close in competitive proximity, and the buying habits of consumers lead to a conclusion that there is a likelihood of confusion as to the source of publication of *Celebrity* magazine. Each of these factors weigh in Time's favor. It is fair to conclude therefore, that there is a likelihood that the public will be confused and that Time has proven its cause of action for the infringement of its trade dress.

Irreparable Injury

[5] As indicated earlier to obtain a preliminary injunction, Time must establish that *Celebrity*'s use of its cover format will cause irreparable injury. Where a party seeks a preliminary injunction in a trademark infringement case, irreparable harm is demonstrated by a showing of likelihood of confusion as to the source or sponsorship of the magazine. *Home Box Office v. Showtime/The Movie Channel*, 832 F.2d 1311, at 1314 [4 USPQ2d 1789, 1791]. Here Time has shown a likelihood of confusion sufficient to meet the showing of irreparable harm.

Balance of the Hardships

Globe has printed 125,000 copies of the 1989 issue, approximately half of their regular level of production. However, they have only published one issue with the new cover and there are numerous other cover formats from which they could choose a new one. Further, given the finding of irreparable harm to Time and likelihood of confusion among consumers, allowing Globe to continue to use its current cover format could cause serious harm to Time. Therefore, the balance of hardships weigh in Time's favor.

Conclusion

Time has established a trade dress in the *People* cover format which includes the condensed white lettering, and the display of the logo with a contrasting colored border. Globe has not shown that there is no likelihood of confusion, thus Time is likely to succeed on the merits of their claim of trademark infringement. Further, Time has shown that they will suffer irreparable harm if the injunction is not granted and that the balance of hardships tips in their favor. Further, for the reasons set forth above, Time's motion for a preliminary injunction is granted.

It is so ordered.

Patent and Trademark Office
Board of Patent Appeals and Interferences

Ex parte Gray

No. 88-0437

Decided August 17, 1988, and January 17, 1989
Released March 14, 1989

PATENTS

1. Patentability/Validity — Obviousness — In general (§115.0901)

Patentability/Validity — Obviousness — Relevant prior art (§115.0903)

Patent and Trademark Office does not have facilities for examining and comparing applicants' claimed human nerve growth factor, which is product-by-process claim, with prior art, and thus applicants had burden of persuasion to make some comparison between materials in order to establish unexpected properties for claimed factor, and applicants, having failed to do so, cannot contend on appeal that any doubt as to difference between two materials should be resolved in favor of patentability, since obviousness does not require absolute predictability.

2. Patentability/Validity — Obviousness — Relevant prior art (§115.0903)

Applicant can be required to prove that prior art products do not necessarily or inherently possess characteristics of claimed product, and thus applicants, on appeal of rejection in which issue is whether prior art factor is identical or patentably indistinct from that of material on appeal, have burden of showing that inherency is not involved.

3. Patentability/Validity — Obviousness — Evidence of (§115.0906)

Mere conclusory statements in publication item are no more probative of non-obviousness than such statements would be in applicant's specification, and, even if such unverified statements were to be considered as those of expert in art, such statements would be inadequate in view of lack of any factual supporting evidence.

4. Patentability/Validity — Obviousness — In general (§115.0901)

Mere purity of claimed compound does not render substance unobvious.

5. Patentability/Validity — Obviousness — Relevant prior art (§115.0903)

Patentability/Validity — Adequacy of disclosure (§115.12)

Applicants whose claims for human nerve growth factor synthesized through use of recombinant DNA technology were rejected for obviousness must, in order to raise question of non-enablement of prior art, provide at very least declaration by person having ordinary skill in subject art that no method was known to that person prior to claimed invention whereby claimed material might have been synthesized.

Appeal from rejection of claims (Howard E. Schain, primary examiner, G.D. Draper, examiner).

Application for patent filed by Alane M. Gray and Axel Ullrich, serial no. 471,962, on March 3, 1983. From examiner's decision rejecting claims, applicants appeal. Affirmed.

Max D. Hensley, San Francisco, Calif., for appellants.

Before Pellman, Winters, and W. Smith, examiners-in-chief.

Pellman, examiner-in-chief.

This is an appeal from the examiner's decision finally rejecting claims 1, 11, 12, 13 and 15 through 18, remaining claims 19 and 20 having been withdrawn from consideration by the examiner. However, since, by amendment, claims 13, 15 and 16 were canceled, the claims before us for consideration are 1, 11, 12, 17 and 18.

The subject matter on appeal involves the human nerve growth factor β-NGF, identified by the particular amino acid sequence and being free from other proteins of human origin (claim 1). The invention also includes pharmaceutical compositions containing said nerve factor (claims 11 and 12) and said human nerve factor in which the amino acid sequence is preceded by a methionyl group (claims 17), as well as a composition containing the factor of claim 17 (claim 18). The particular human nerve factor of the present invention has been synthesized through the use of recombinant DNA technology and thus, is free from human proteins that would otherwise be expected to contaminate the composition. To describe the invention in greater detail and illustrate the claims on appeal, a copy of claim 1 is appended to this decision.

For evidence of obviousness, the references identified below are cited by the examiner.

Goldstein et al. (Goldstein) "Isolation of Human Nerve Growth Factor From Placental Tissue," Neurochemical Research *3*, 175-183 (1978)

Walker et al. (Walker), "Human Nerve Growth Factor: Lack of Immunocrossreactivity with Mouse Nerve Growth Factor," Life Sciences *26*, 195-200 (1980)

All of the claims stand rejected for being unpatentable (35 U.S.C. 103) in view of Goldstein or Walker. The examiner, at page 2 of the answer states that:

"Each of these prior art discloses human β-NGF that appears to be the same as that claimed wherein such was isolated from human placental tissue, versus the claimed β-NGF that was produced by recombinant techniques."

In the sentence bridging pages 2 and 3 of the answer, the examiner notes that the sequencing of a protein does not make the protein different, but merely constitutes a further characterization of the known material.

In response to the examiner's arguments, beginning at page 5 of the brief appellants

set forth their own arguments. Appellants apparently divide their argument into three points. The first is that the human β-NGF of the references is not inherently that of appellants. Second, appellants contend that the reference human nerve growth factor is not free from other human proteins. Finally, we are told that the cited prior art does not teach or suggest methionyl N-terminal human β-NGF.

At page 6 of their brief, appellants refer to the publication edited by Black, *Cellular and Molecular Biology of Neuronal Development*, Plenum Press, New York, Chapter 20, Breakefield et al, pages 309-328 (1984). Appellants refer specifically to page 310, wherein the author states that:

> "To establish whether patients with dysautonomia make an altered form of β-NGF, it is necessary to characterize the human form of this protein. This has been difficult, and although there are a number of reports on preliminary identification of a human NGF-like molecule (Goldstein *et al.*, 1978; Walker *et. al.*, 1980), no one has conclusively demonstrated its presence."

Appellants rely upon the foregoing as evidence that the Goldstein and Walker reports are merely preliminary and inconclusive.

At page 8 of their brief, appellants focus on the difference in the interpretation of Walker by the examiner vis-a-vis that by appellants as to the lack of immunological cross-reactivity between the art human β-NGF and murine β-NGF. Appellants conclude that the point is not whose theory is right, but contend that "when legitimate disputes arise about polypeptide identity they must-in view of prior board precedent, be resolved in appellants' favor. Rejections cannot be properly maintained on "maybe" references."

Beginning at page 9 of their brief, appellants raise the question of the purity of the claimed human β-NGF as compared with that of the references. Appellants explain that, due to the method of preparation, their nerve growth factor is free from any other human proteins. At page 10 of the brief, appellants query "why would the art be motivated to attempt to further purify human "β-NGF" beyond the level reported by Goldstein *et al* and Walker *et al*? If so motivated, does the art reasonably teach one of ordinary skill how to do so?"

In connection with the foregoing, at page 10 of the brief, appellants suggest that even if art were applied showing recombinant methods for synthesizing proteins, it would be clear from their discussion that more than a conventional recombinant method was involved in the preparation of the claimed human nerve growth factor.

At page 11 of their brief, appellants discuss the methionyl N-terminal human nerve growth factor. We are informed that "no reference of record teaches any reason for wanting to make methionyl β-NGF, and no reference teaches how to do so even if that was an objective. With regard to the therapeutic formulation of claim 18, there is no teaching or suggestion as to what sort of biological activity to reasonably expect from the methionyl N-terminal variant."

Although due consideration has been given to the opposing arguments and supporting evidence of appellants and of the examiner, we are unpersuaded of reversible error in the examiner's rejection, which will be sustained.

While the present claims are drafted in the form of a compound or a composition, the rationale underlying appellants' arguments is founded on the proposition that the claims are directed to a product-by-process. In any event, we are convinced that the legal philosophy employed in rejections involving products-by-process should be employed with respect to the claims before us. That is, insofar as we can observe, the difference between the material of Goldstein and of Walker and that claimed by appellants herein resides in the method of obtaining the human growth factor. The prior art material is recovered from natural sources and purified, while appellants' is produced by recombinant DNA methodology. However, the dispositive issue before us is whether the claimed factor exhibits any unexpected properties compared with that described by the cited publication items.

To answer the foregoing question, we turn to the decision in *In re Brown*, 59 CCPA 1036, 459 F.2d 531, 173 USPQ 685 (1972) wherein, at 59 CCPA 1041, Judge Baldwin, delivering the court's opinion, explains:

> "We are therefore of the opinion that when the prior art discloses a product which reasonably appears to be either identical with or only slightly different than a product claimed in a product-by-process claim, a rejection based alternatively on either section 102 or section 103 of the statute is eminently fair and acceptable. As a practical matter, the Patent Office is not equipped to manufacture products by the myriad of processes put before it and then obtain prior art products and make physical comparisons therewith."

[1] Consistent with the court's holding, we find that, in the present case, the Office does not have the facilities for examining and comparing appellants' growth factor with that disclosed by Walker and by Goldstein. It is therefore entirely proper that appellants should have shouldered their burden of persuasion and made some comparison between the two materials to establish unexpected properties for the claimed factor. Having failed to do so, appellants are in a poor position now to contend that any doubt as to the difference between the two materials should be resolved in *favor* of patentability. Appellants do not inform us of the legal basis for their conclusion that this Board has held that doubt should be resolved in favor of an applicant and we are aware of no such recent decision. On the other hand, our reviewing tribunal, the United States Court of Customs and Patent Appeals in *In re Mixon*, 59 CCPA 1996, 470 F.2d 1374, 176 USPQ 296 (1973), responsive to Chief Judge Worley's discussion of the "rule of doubt", Judges Rich, Almond, Baldwin and Lane, in their concurring opinion state:

> "Since we have not been following any 'rule of doubt' policy and since that question is not involved in the present case we do not agree with the additional comments of the author."

In fact, rather than resolving doubt in favor of the applicant, the court has often held that obviousness does not require absolute predictability. See the decisions in *In re Merck and Company, Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986) and *In re Lamberti*, 545 F.2d 747, 192 USPQ 278 (CCPA 1976).

[2] At page 5 of their brief, appellants contend that human β-NGF, as described by Walker or Goldstein, is not "inherently" that of appellant. However, this has not been established. Appellants' attention is invited to the decision in *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977), wherein the court held that the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. Accordingly, since the issue in the present appeal is whether the prior art factor is identical or patentably indistinct from that of the material on appeal, appellants have the burden of showing that inherency is not involved.

[3] Following the court's guidance in *In re Johnson*, 747 F.2d 1456, 223 USPQ 1260 (Fed. Cir. 1984), we have weighed the examiner's evidence of obviousness against appellants' countervailing evidence to determine whether the claims are patentable notwithstanding the references of record. It appears that appellants rely heavily upon the publication item by Breakefield, particularly pages 310 and 311. We are well aware that the author dismisses the "preliminary identification of a human β-NGF-like molecule" by Goldstein and Walker and concludes that no one has conclusively demonstrated its presence. Nevertheless, we do not interpret the subjective statement by Breakefield as adequate to overcome the specific findings reported by Goldstein and Walker. Mere conclusory statements in a publication item are no more probative of nonobviousness than would be said statements in appellants' specification. Compare *In re D'Ancicco*, 59 CCPA 748, 452 F.2d 1060, 172 USPQ 241 (1972). Moreover, even if we were to consider the unverified statements in the publication article as those of an expert in the art, the statements would be inadequate because of the lack of factual supporting evidence. Compare *In re Grunwell*, 609 F.2d 486, 203 USPQ 1055 (CCPA 1979).

The present situation is somewhat similar to that confronting the court in *Scripps Clinic & Research Foundation v. Genentech Inc.*, ——— F.Supp. ———, 3 USPQ2d 1481 (DC NCalif 1987) wherein human blood-clotting factor VIII:C was involved. At page 3 USPQ2d 1487, the court points out that:

> Scripps also alleges infringement of product claims 24 through 29 covering concentrated preparations of "human Factor VIII:C.
>
> According to Scripps, these claims cover preparations, in the specified ranges of purity and potency, of Factor VIII:C with the functional and structural characteristics of the protein as it occurs naturally in humans.[6] Genentech, on the other hand, argues that Scripps' claims are limited to Factor VIII:C derived from human blood plasma. The issue posed is whether the asserted product claims must be interpreted to apply solely to concentrates of Factor VIII:C derived directly from human blood plasma or whether they extend also to other concentrates of Factor VIII:C having the same characteristics as those derived from human blood plasma.

In the paragraph bridging pages 1488–1489, the court explains that:

"the excerpts quoted by Genentech from deposition testimony of Drs. Katzmann and Zimmerman, Scripps' experts, that 'human' means 'obtained from human blood', are not probative on the issue of interpreting the claims. Dr. Katzmann's answer related to Factor V, not Factor VIII, and Dr. Zimmerman's answer did not purport to give an interpretation of the particular claim language. Human factor VIII:C as claimed in the patent therefore applies to any Factor VIII:C preparation, regardless of how produced, having the same material structural and functional characteristics as the plasma-derived preparation."

We are convinced that our decision herein is completely consistent with and supported by the above-noted holding of the District court in Northern California.

In the interest of completeness, we call attention to two other decisions that appear relevant to our present holding. The first of these is *In re Bergstrom*,[1] 57 CCPA 1240, 427 F.2d 1394, 166 USPQ 256 (1970), involving a rejection of certain pure prostaglandin compounds for not being novel in light of the material from which it was extracted. At page 57 CCPA 1250, the court held as follows:

"We need not decide the merits of that matter, for the fundamental error in the board's position, as we see it, is the analysis and answer it gave to the sole issue it accurately posed — 'whether the *claimed* pure materials are *novel* as compared with the *less pure* materials of the reference.' [emphasis supplied.] It seems to us that the answer to that question is self-evident: by definition, pure materials necessarily differ are the only ones existing and available as a standard of reference, as seems to be the situation here, perforce the 'pure' materials are 'new' with respect to them."

The other decision relevant to the facts before us, is In re *Wakefield*, 57 CCPA 959, 422 F.2d 897, 164 USPQ 636 (1970). In the *Wakefield* case, the claimed subject matter was synthetic rubber, while the prior art showed the corresponding naturally occurring product. At page 57 CCPA 966, Judge Lane, speaking for the court, disagreed with the board that the word "synthetic" as used in the claims would be applicable to purified natural product. In delivering the court's opinion, Judge Lane held that:

"we now turn to the examiner's view adopted by the Board, that the synthetic product is so similar to the natural product, purified to the extent allegedly shown in Davis, as to be '*prima facie* obvious'. We would agree with this conclusion as a tentative one based on similarity of structure and gross characteristics. However, such tentative conclusions of obviousness are rebutted in those instances where there was, at the time the invention was made, no known or obvious method of making the claimed composition, or where the claimed composition is found to possess unexpected characteristics. At least the first situation is present in the case before us, since it cannot be said that a method of making the claimed synthetic product would be known or obvious from Davis."

Although we acknowledge that our holding in the present case appears to be in conflict with the court's limited holding in the Wakefield appeal, we are convinced that our decision is consonant with the overwhelming weight of current patent jurisprudence involving questions of the type posed by appellants. Moreover, we point out that no objective evidence has been provided establishing that no method was known to those skilled in this field whereby the claimed material might have been synthesized. Therefore, although we have weighed all of the evidence and legal authorities, both pro and con, concerning the patentability of the claims on appeal, we find that the evidence and the weight of legal authority compels an affirmance of the examiner's rejection.

With respect to claims 17 and 18, the mere presence of a single methionyl moiety in a sequence of over 100 amino acids would not have been expected to alter the properties of the compound in a significant respect, in the absence of evidence to the contrary. It is our view that a minor inactive substituent on an otherwise unpatentable complex compound will not necessarily impart patentability to said compound. Thus, since we find claims 17 and 18 to be directed to an unpatentable modification of the compound to which the remaining claims are directed, these claims are held to be properly rejected for the same reasons as claims 1, 11 and 12.

For the reasons expressed above and those set forth in the answer, the examiner's decision rejecting claims 1, 11, 12, 17 and 18 is affirmed.

37 CFR 1.136(a) does not apply to the times for taking any subsequent action in connection with this appeal.

AFFIRMED.

[1] Cited by the court in footnote 6 in the *Scripps Clinic* decision.

ON REQUEST FOR RECONSIDERATION

January 17, 1989

Appellants request us to reconsider our holding mailed August 17, 1988 in which we affirmed the examiner's decision rejecting claims 1, 11, 12, 17 and 18.

At page 7 of their request, appellants state that there is no basis in the art of record for reasonably predicting that human beta-NGF could be produced by recombinant host cells. However, appellants appear to misapprehend the basis for our decision. It is our explicit holding that the product to which appellants' claims are directed would have been expected to be the same or substantially the same as that of the human nerve growth factor isolated by Goldstein and by Walker. At page 180 of his article, Goldstein states, under "DISCUSSION" that "we have demonstrated that human placental cotyledons are a suitable source for the purification of human NGF." Likewise, at page 195 of the other publication item cited by the examiner, Walker, in the "Summary", reports "Human B-nerve growth factor (hNGF) was purified from term human placenta." In the last six lines of the first paragraph at page 195, Walker discloses that:

Recently, Goldtein and coworkers (14) isolated and purified the biologically active β subunit of NGF from term human placenta. The present report confirms the presence of human β-NGF (hNGF) in term human placenta and reports the lack of immonocross-reactivity between mouse (mNGF) and hNGF using 6 different antisera to mNGF.

Beginning at page 5 of our decision, we pointed out that the legal principles enunciated in cases involving product-by-process claims are considered to be applicable herein. In support thereof, we cited the decision in *In re Brown*, which clearly explains the basis for our holding. That is, where the product disclosed in the prior art reasonably appears to be either identical with or slightly different from a product claimed by an applicant, there is pragmatic justification for placing the burden of going forward on the applicant. Furthermore, at page 10 of our decision, after acknowledging the apparent conflict between our opinion and the court's holding in *In re Wakefield*, we asserted that our decision is consonant with the overwhelming weight of current patent jurisprudence. Nevertheless, at page 16 of the request for reconsideration, appellants contend that they can find *no* decisions that support our position. Accordingly, appellants' attention is invited to the decisions in *In re Thorpe*, 777 F.2d 695, 227 USPQ 964 (Fed. Cir. 1985); *In re Marosi*, 710 F.2d 799, 218 USPQ 289 (Fed. Cir. 1983); *In re Fitzgerald*, 619 F.2d 67, 205 USPQ 594 (CCPA 1980); *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Avery*, 518 F.2d 1228, 186 USPQ 161 (CCPA 1975); *In re Fessman*, 489 F.2d 742, 180 USPQ 324 (CCPA 1974); and *In re Luck*, 476 F.2d 650, 177 USPQ 523 (CCPA 1973), just to name a few. Additionally, for appellants' convenience, we quote the following passage from *In re Best*, 195 USPQ 433-434, cited at page 7 of our decision:

Where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. See In re Ludtke,[2] supra. Whether the rejection is based on 'inherency' under 35 USC 102, on 'prima facie obviousness' under 35 USC 103, jointly or alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products.

[4] Having disposed of the arguments adverting to the product-by-process rationale, we note that, beginning at page 12 of their petition, appellants contend, in effect, that the pure form of a known substance may be patentable. To support their position, appellants refer to the famous "aspirin" case, cited at page 13 of the petition. Nonetheless, the *mere* purity of a compound, in itself, does not render the substance unobvious. Compare the decisions in *Ex parte Hartop*, 139 USPQ 525 (Bd.App. 1962); *Ex parte Steelmand*, 140 USPQ 189 (Bd.App. 1962); *In re Mehta*, 52 CCPA 1615, 347 F.2d 859, 146 USPQ 284 (1965); and *In re Avery*, *supra*. Furthermore, with respect to the decision in *In re Williams*, cited at page 14 of the petition, appellants' attention is invited to the subsequent decision in *In re Adamson*, 47 CCPA 839, 275 F.2d 952, 125 USPQ 233 (1960), which shows that the *Williams* decision resulted from the absence of relevant available evidence and does not represent a controlling rule of law. Also compare *In re*

[2] 58 CCPA 1159, 441 F.2d 660, 169 USPQ 563 (1971).

Anthony, 56 CCPA 1443, 414 F.2d 1383, 162 USPQ 594 (1969).

[5] At page 11 of our decision, we noted that no objective evidence had been provided establishing that a method was unknown to those skilled in the field whereby the claimed material might have been synthesized. In response thereto, at page 6 of the petition, appellants complain that this improperly shifts the burden of proof to them and places them in the untenable position of having to prove a negative of enormous scope. We disagree. Rather, we are of the opinion that, to raise the question of nonenablement, appellants must, at the very least, provide a declaration by a person having ordinary skill in the subject art that no method was known to him prior to the claimed invention whereby the claimed material might have been synthesized. In this connection, attention is invited to the decision in *In re Collins*, 59 CCPA 1170, 462 F.2d 538, 174 USPQ 333 (1972). It will be noted that in said decision, not only was an affidavit required, but the court agreed with the board that the submitted affidavit failed to establish that there was no known or obvious way to make heat exchangers falling within the scope of the appealed claims. However, in the interest of reducing the issues in this case, we will agree, *arguendo*, that the only methods for obtaining human nerve growth factor, other than that of appellants, are those disclosed by Goldstein and by Walker, of record.

At page 11 of the petition, appellants again rely upon Breakefield as casting doubt on the identity of the materials reported in the cited references. At page 12 of said petition, appellants state that the Breakefield reference "serves as expert testimony". We are then requested to provide our own evidence to counter the Breakefield et al. findings. Nevertheless, we will decline appellants' invitation.

The reason for requiring evidence in declaration or affidavit form is to obtain the assurances that any statements or representations made are correct, as provided by 35 U.S.C. 25 and 18 U.S.C. 1001. To permit the Breakefield publication, coauthored by one of the appellants herein, to substitute for expert testimony would circumvent the guarantees built into the statute by Congress. Accordingly, it is clear that we have no duty to offer evidence to counter the statements made by Breakefield. Rather, we are charged with the obligation of balancing all of the cited evidence of obviousness against the submitted evidence of non-obviousness. See the paragraph bridging pages 7 and 8 of our decision. In so weighing the evidence, we determined that the Breakefield publication item was inadequate to counterbalance the factual findings in the two publication items provided by the examiner. Consequently, we are convinced that our decision in the present case fully complies with the requirements of the statute and 37 C.F.R. 1.196(a).

Focusing now on claims 17 and 18, we observe that appellants, beginning at page 17 of their petition, again separately argue the patentability of human methionine beta-NGF. However, appellants have failed to respond to our finding that the protein containing the terminal methionyl group is substantially identical in structure to that purified by Goldstein and Walker. Since the decisions, such as *In re Brown*, of record, and *In re Best*, of record, agree that the disclosure of a *substantially* identical material in a prior art reference is sufficient to establish a prima facie case of obviousness and shift the burden of proof to appellants, we find no reason to arrive at a different conclusion.

At page 21 of their request, appellants acknowledge that the exhibits accompanying said request are newly cited and have not been considered by the examiner. Nonetheless, appellants request us to consider the references to economize our time. Ignoring the submitted publication items, appellants caution, will insure that said items will be presented in a continuing application. However, the same reasoning might be employed to extend the prosecution in any application handled by an examiner or to dispute any decision rendered by this Board. Since there must be an end to prosecution in any particular case, the mere possibility of further prosecution in a continuing application is insufficient reason for us to consider the publications cited by appellants herein. Compare *In re Fessman*, *supra*.

Although, to the extent indicated, we have reconsidered our decision, we decline to make any changes therein.

DENIED.

APPENDIX

1. Human β-NGF comprising the amino acid sequence ser-ser-ser-his-pro-ile-phe-his-arg-gly-glu-phe-ser-val-cys-asp-ser-val-ser-val-trp-vil-gly-asp-lys-thr-thr-ala-thr-asp-ile-lys-gly-lys-glu-val-met-val-leu- gly-glu-val-asn-ile-asn-asn-ser-val-phe-lys-gln-tyr-phe-phe-glu-thr-lys- cys-arg-asp-pro-asn-pro-val-asp-ser-gly-cys-arg-gly-ile-asp-ser-lys-his-trp-asn-ser-tyr-cys-thr-thr-thr-his-thr-phe-val-lys-ala-leu-thr-met-asp-gly-lys-gln-ala-ala-trp-arg-phe-ile-arg-ile-asp-thr-ala-cys-val-cys-val-leu-ser-arg-lys-ala-val-arg and which is free of other proteins of human origin.

District Court, N.D. California

Bausch & Lomb Inc. v. Barnes-Hind/Hydrocurve Inc.

No. C 83-20283 RPA

Decided March 22, 1989

PATENTS

1. Patentability/Validity — Obviousness — Relevant prior art (§115.0903)

Relevant prior art for determining obviousness of laser-engraved soft contact lens invention consists primarily of patent for laser apparatus for cutting holes in hard contact lenses and patent disclosing use of laser to engrave plastic surface of printing plate, plus development of laser technology between 1968 and 1976.

2. Patentability/Validity — Obviousness — Combining references (§115.0905)

Patent for laser-engraved soft contact lens is not obvious in view of prior patent on laser apparatus for fenestration of hard contact lenses in combination with prior patent disclosing use of laser to engrave plastic surface of printing plate, since latter patent "taught away" from using process on materials suitable for soft contact lenses, and since Court of Appeals for the Federal Circuit held, as law of case, that one skilled in art would not have construed laser fenestration teachings of former patent as applying to soft contact lenses.

3. Patentability/Validity — Obviousness — Secondary considerations generally (§115.0907)

Evidence of secondary considerations is not persuasive as to non-obviousness of patent for laser-engraved soft contact lens, since evidence of copying is inconclusive, since need to use automatic engraving was not long-felt but rather arose in early 1980s as result of growth in sales of soft contact lenses, and since patented marking system did not bring patent holder commercial success.

4. Infringement — Literal infringement (§120.05)

Plaintiff's patent for laser-engraved soft contact lens is infringed by defendants' contact lenses, since issue of infringement involves only whether surface of defendants' lens surrounding laser marks is "smooth," since specification indicates that "smooth" means absence of ridges that would scratch eye or eyelid, and since defendants' lenses do not inflame or irritate wearers' eyes.

Particular patents — General and mechanical — Contact lenses

4,194,814, Fischer, McCandless and Hager, transparent opthalmic lens having engraved surface indicia, valid and infringed.

On remand from the U.S. Court of Appeals for the Federal Circuit; 230 USPQ 416.

Action by Bausch & Lomb Inc. against Barnes-Hind/Hydrocurve Inc. and Barnes-Hind International Inc., for patent infringement. On remand from decision vacating judgment for defendants. Judgment for plaintiff.

Laurence H. Pretty and Craig S. Summers, of Pretty, Schroeder, Brueggemann & Clark, Los Angeles, Calif.; Anne L. Enea, of Ferrari, Alvarez, Olsen & Ottoboni, San Jose, Calif., for plaintiff.

John M. Calimafde, Paul H. Blaustein, and Dennis J. Mondolino, of Hopgood, Calimafde, Kalil, Blaustein & Judlowe, New York, N.Y.; Douglas K. Tribble, of Pillsbury, Madison & Sutro, San Jose, for defendant.

Aguilar, J.

FINDINGS OF FACT AND CONCLUSIONS OF LAW FOLLOWING REMAND

This patent infringement case returns to this Court on remand from the United States Court of Appeals for the Federal Circuit. The circuit vacated the judgment entered after trial as improper for the following reasons:

(1) this Court did not explicitly set forth in its Order the presumption of validity that it awarded the patent under 35 U.S.C. §282;

(2) the Court did not set forth factual findings on the four inquiries mandated by *Graham v. John Deere Co.*, 383 U.S. 1, 17 [148 USPQ 459, 467] (1966); and

(3) the circuit court found this Court engaged in improper claim construction.

scribed before. As a result, different p-n diodes manifest varying degrees of discharge resulting in a fluctuating current in the semiconductor as the beam scans successive p-type regions recharging them to the full target voltage.

The difference between Buck's p-n junction structure and Desvignes' two p-n junction structure can be seen from the above figure illustrating the Desvignes device.

Throughout, Buck's specification speaks of "diode," "diodes," "p-n diodes," "diode array," "target diode array," "diode target array," "diode junction," "diode capacitance," "target surface diodes," and "regional rectifying diode array."

Finally, it is to be noted that the record shows that the examiner allowed the Buck patent notwithstanding citation to the original Desvignes patent. *See* McCutchen v. Oliver, 367 F.2d 609, 616, 54 CCPA 756, 765 (1966).

Accordingly, I would hold that Desvignes has not met his burden of proving that his disclosure supports the count and reverse the decision of the board.

Application of Gerhard FESSMANN.
Patent Appeal No. 9121.

United States Court of Customs and Patent Appeals.
Jan. 10, 1974.

Appeal was taken from decision of the Patent Office Board of Appeals, Serial No. 850,980, affirming examiner's rejection of claim relating to a liquid composition used for imparting a smoked flavor to foodstuffs, obtained by treating sawdust with superheated steam. The Court of Customs and Patent Appeals, Almond, Senior Judge, held that product-by-process claim was properly rejected as obvious where the only characterization of the composition of the product was that it had a low content of carcinogens and where prior art references described compositions, obtained by other processes, having little or no carcinogen content.

Affirmed.

1. Patents ⇐32

The patent office bears a lesser burden of proof in making out a case of prima facie obviousness of product-by-process claim than would be the case when a product is claimed in the more conventional fashion.

2. Patents ⇐18

Product-by-process claim of application with respect to invention relating to a liquid composition used for imparting a smoked flavor to foodstuffs, obtained by treating sawdust with superheated steam, was properly rejected as obvious, where the only characterization of the composition of the product which appeared in the specification was that it had a low content of carcinogens, and where prior art references described compositions, produced by other processes, having little or no carcinogen content. 35 U.S.C.A. § 103.

3. Patents ⇐32

Where patent office made out a claim of prima facie obviousness of a product-by-process claim, burden shifted to applicant to demonstrate the unobvious character of his claimed invention over the cited references.

4. Patents ⇐110

Patent office board of appeals was within its authority when it rejected belated offer of evidence made in request for reconsideration of rejection of claim, where the board had made no new rejection and applicant was on notice as to the basis of the rejection by reason of the examiner's rejection on the same ground.

Edward W. Goldstein, Washington, D. C., attorney of record, for appellant.

Joseph F. Nakamura, Washington, D. C., for Commissioner of Patents, Jack E. Armore, Washington, D. C., of counsel.

Before MARKEY, Chief Judge, RICH, LANE and MILLER, Judges, and ALMOND, Senior Judge.

ALMOND, Senior Judge.

This is an appeal from the decision of the Patent Office Board of Appeals affirming the examiner's rejection, under 35 U.S.C. § 103, of claim 1 in appellant's application [1] entitled "Liquid Smoke." We affirm.

The Invention

The invention relates to a liquid composition used for imparting a smoked flavor to foodstuffs such as meat, sausage and cheese. The composition is obtained by treating sawdust with superheated steam. The precise nature of the invention and the method for making it will be more apparent from a consideration of claim 1, reproduced below, since the claim is in the product-by-process format.

1. A liquid smoke product comprising at least a portion of the material resulting from a process of:

(a) contacting particulate wood solids with superheated steam having a temperature of at least 180°C. to effect a thermal decomposition of said wood solids to form a smoking fluid;

(b) withdrawing the smoking fluid from the wood solids; and

(c) liquifying the smoking fluid to produce said liquid smoke product which may be stored and subsequently applied in the treatment of foodstuffs.

Other than the process by which it is made, the only characterization of the composition which appears in the specification is that it has a low content of carcinogens. In particular, it contains 0.2–0.3 micrograms of 3,4-benzopyrene per liter. The record makes it clear that prior art "liquid smoke" compositions are complex mixtures of the chemical compounds which can be derived from wood. These mixtures defy simple characterization and this fact presumably accounts for the use of a product-by-process claim.

Rejection

The examiner rejected claim 1 as being obvious within the meaning of § 103 in view of each of the following give patents:

Chase	511,288	Dec.	19, 1893
Guinot	2,454,649	Nov.	23, 1948
Hollenbeck	3,106,473	Oct.	8, 1963
Taylor	3,152,914	Oct.	13, 1964
Miler et al. (Miler)	3,445,248	May	20, 1969
	(Filed	Apr.	7, 1965)

All of these references are directed to liquid smoke compositions and/or their method of preparation. However, none of these is obtained by treating sawdust with superheated steam. For the purposes of our decision, it will suffice to discuss but two of these references, Hollenbeck and Miler, which in our view are the most pertinent.

Hollenbeck discloses that it was known to the prior art that a liquid smoke composition could be obtained from wood. In these processes the smoke can be generated by burning wood in a limited amount of air or by destructively distilling wood. The resulting product can be condensed to a liquid or dissolved in water to make the liquid smoke. Hollenbeck prefers to use the method in which the wood is burned since this produces lesser amounts of phenolic and hydrocarbon compounds. Although some phenolics are desired, if their content is too high a bad taste is imparted to the foodstuff. Among the hydrocarbons produced is 3,4-benzopyrene.

Hollenbeck obtains his product by the countercurrent extraction of the smoke using water as the extractant. The extract is condensed and filtered with cellulose pulp to remove the 3,4-benzo-

1. S.N. 850,980 filed August 18, 1969 as a continuation-in-part of S.N. 515,126 filed December 20, 1965 and now U.S. Patent No. 3,462,282.

pyrene. This results in a content of 3,4-benzopyrene of less than 0.5 parts per billion.

Miler also generates the smoke by burning wood. However, an excess of air rather than limited air is used in the combustion. The smoke is absorbed into an aqueous alkali solution. Miler teaches that carcinogenic compounds such as 3,4-benzopyrene can be removed from the alkali solution by extraction with ethyl ether.

In the examiner's view, appellant failed to show that the liquid smoke obtained by his process possessed unobvious differences from the liquid smoke compositions shown in the prior art. The board agreed, reasoning as follows:

> In the present case, we have searched in vain in appellant's brief for some explicit assertion of unobviously novel properties or characteristics exhibited by his liquid smoke product. However, all that we find is a comparison of his process with the prior art processes. A comparison of that nature does not serve to resolve the issue concerning the patentability of the product. Even a review of appellant's specification is of little assistance to appellant's cause. The specification emphasizes the low 3,4 benzopyrene content of appellant's product * * * but this is not a novel characteristic of a liquid smoke product as evidenced by the Hollenbeck patent * * * and the Miler et al. patent * * *.

Appellant requested reconsideration by the board of its decision. In his request he offered new evidence which was alleged to show that his liquid smoke differed in kind from that known to the prior art and that this difference grows out of his unobvious process.[2] He also offered to provide additional evidence if given time. The board refused to consider the new evidence for the reason that, in its view, the evidence was not timely presented.

OPINION

This court recently made the following observation, in In re Brown, 459 F.2d 531, 59 CCPA 1036 (1972), regarding the patentability of product-by-process claims:

> * * * the lack of physical description in a product-by-process claim makes determination of the patentability of the claim more difficult, since in spite of the fact that the claim may recite only process limitations, it is the patentability of the *product* claimed and *not* of the recited process steps which must be established. We are therefore of the opinion that when the prior art discloses a product which reasonably appears to be either identical with or only slightly different than a product claimed in a product-by-process claim, a rejection based alternatively on either section 102 or section 103 of the statute is eminently fair and acceptable. As a practical matter, the Patent Office is not equipped to manufacture products by the myriad of processes put before it and then obtain prior art products and make physical comparisons therewith. [Emphasis in the original.]

[1, 2] In *Brown*, the court was in effect saying that the Patent Office bears a lesser burden of proof in making out a case of prima facie obviousness for product-by-process claims because of their peculiar nature than would be the case when a product is claimed in the more conventional fashion. We believe the Patent Office satisfied its burden of proof in this case in view of Hollenbeck and Miler.

As the board noted, there is nothing in appellant's specification which suggests that his liquid smoke differs from the prior art compositions other than by its low content of carcinogens. However, Hollenbeck and Miler describe compositions having little or no carcinogen content. Hollenbeck does teach that the method of manufacture can affect the

2. Appellant's patent, U.S. 3,462,282, contains claims directed to a process for obtaining liquid smoke using superheated steam. This patent issued on an application of which the application involved in this appeal is a continuation-in-part.

makeup of a liquid smoke composition. However, there is nothing in the record before the examiner from which he could reasonably have been expected to conclude that appellant's composition differs in kind from those obtained by other inventors solely because it was derived from a process not known to the prior art.

[3, 4] Accordingly, we think the board acted properly when it affirmed the examiner as the burden of proof had shifted to the appellant and this burden was not discharged. It was appellant's duty to present evidence which would demonstrate the unobvious character of his claimed invention over the cited references. No such evidence was offered prior to the reaching by the board of its decision and we think the board was within its authority when it rejected the belated offer of evidence made in appellant's request for reconsideration. The board had made no new rejection and appellant was clearly on notice that the examiner was not persuaded that the composition was unobvious simply because its process of manufacture had been deemed patentable.

For the foregoing reasons, the decision of the board is affirmed.

Affirmed.

Richard J. DUFFY, Petitioner,

v.

Rene D. TEGTMEYER, Acting Commissioner of Patents,
and
Gerald Barnes et al., Respondents.

Special Patent No. 182.

United States Court of Customs and Patent Appeals.

Jan. 10, 1974.

Rehearing Denied Feb. 28, 1974.

Proceeding on petition for writ of mandamus seeking to reverse decision of Chairman of Board of Patent Interferences and of the acting commisioner deferring consideration of motion to delete a party as an inventor to final hearing. The Court of Customs and Patent Appeals, Rich, J., held that issuance of writ was not necessary or appropriate in aid of jurisdiction since the loser would have an appeal from a decision on priority.

Writ denied.

1. Patents ⇐113(1)

Court of Customs and Patent Appeals was not without jurisdiction to issue writ of mandamus in aid of its jurisdiction in interference merely because no appeal had been taken from a final decision on priority; the fact that there had not been a decision on priority and no appeal therefrom went to the question whether the court ought to exercise jurisdiction because the issuance of a writ of mandamus would be necessary or appropriate in aid of its jurisdiction and agreeable to the usages and principles of law. 28 U.S.C.A. § 1651(a).

2. Mandamus ⇐1, 28

Writ of mandamus is an extraordinary remedy to be exercised in exceptional circumstances and, in a case involving a discretionary action, only where there has been a clear abuse of discretion. 28 U.S.C.A. § 1651(a).

3. Patents ⇐113(1)

Court of Customs and Patent Appeals has jurisdiction to review decisions of the commissioner of patents on questions which are ancillary to priority in the context of an interference. 35 U.S.C.A. § 116; Patent Office Practice Rules, rules 45(b), 181(g), 231(a)(5), 35 U.S.C.A. App.

4. Patents ⇐113(1)

Writ of mandamus reversing decision of commissioner of patents deferring to final hearing consideration of motion to remove party as a named inventor in the involved application would not be necessary or appropriate in aid of court's jurisdiction since the decision was of an interlocutory nature and with-

ant as well as the public interest, the Commission abuses its discretion by declining to release the bond merely because of sales by a respondent of goods known to the complainant at the time of the agreement.

Biocraft also makes other arguments which we need not address.

CONCLUSION

The Commission's denials of Biocraft's requests for return or cancellation of bonds posted pursuant to the Temporary Cease and Desist Order issued January 10, 1990, were an abuse of discretion. Its order is therefore

REVERSED.

In re Mark A. VAECK, Wipa Chungjatupornchai and Lee McIntosh.

No. 91-1120.

United States Court of Appeals, Federal Circuit.

Oct. 21, 1991.

Inventor sought patent for claimed invention directed to use of genetic engineering techniques for production of insecticidal proteins. The United States Patent and Trademark Office Board of Patent Appeals and Interferences affirmed an examiner's rejection of certain claims, and appeal was taken. The Court of Appeals, Rich, Circuit Judge, held that: (1) patent application was improperly rejected on ground of prima facie obviousness, and (2) patent application was properly rejected to extent that claims were too general to enable person skilled in art to make and use claimed invention without undue experimentation.

Affirmed in part, reversed in part.

Mayer, Circuit Judge, dissented and filed opinion.

1. Patents ⇐314(5)

Obviousness of invention for which patent is sought is legal question which court independently reviews, though based upon Patent and Trademark Office's underlying factual findings, which court reviews under clearly erroneous standard. 35 U.S.C.A. § 103.

2. Patents ⇐16(2)

In reviewing rejection of invention for patent as obvious in view of combination of prior art references, court considers whether prior art would have suggested to those of ordinary skill in art that they should make claimed composition or device, or carry out claimed process, and whether prior art would also have revealed that in so making or carrying out, those of ordinary skill would have reasonable expectation of success; both suggestion and reasonable expectation of success must be found in prior art, not in applicant's disclosure. 35 U.S.C.A. § 103.

3. Patents ⇐16.25

Patent application for genetic engineering techniques for production of insecticidal proteins was improperly rejected on ground of prima facie obviousness; prior art did not disclose or suggest expression in cyanobacteria of chimeric gene encoding insecticidally active protein, or convey to those of ordinary skill reasonable expectation of success in doing so. 35 U.S.C.A. § 103.

4. Patents ⇐99

To be patentable, specification of patent must enable any person skilled in art to which it pertains to make and use claimed invention without undue experimentation. 35 U.S.C.A. § 112.

5. Patents ⇐99

Patent application for using genetic engineering techniques to produce insecticidal proteins was properly rejected to extent that claims were too general to enable person skilled in art to make and use claimed invention without undue experimentation;

claim referred to use of cyanobacteria in general as host organism, despite fact that cyanobacteria were diverse and relatively poorly studied group of organisms, comprising some 150 different genera, with successful use of any one type in manner called for in invention being unpredictable. 35 U.S.C.A. § 112.

6. Patents ⇐99

Although patent applicants are not required to disclose every species encompassed by their claims, even in unpredictable art, in order to satisfy enablement requirement for patentability, there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and how to use invention as broadly as it is claimed. 35 U.S.C.A. § 112.

Ian C. McLeod, Ian C. McLeod, P.C., Okemos, Mich., argued for appellant.

Teddy S. Gron, Associate Sol., Office of the Sol., of Arlington, Va., argued for appellee. With him on the brief were Fred E. McKelvey, Sol. and Richard E. Schafer, Associate Sol.

Before RICH, ARCHER, and MAYER, Circuit Judges.

RICH, Circuit Judge.

This appeal is from the September 12, 1990 decision of the Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences (Board), affirming the examiner's rejection of claims 1–48 and 50–52 of application Serial No. 07/021,405, filed March 4, 1987, titled "Hybrid Genes Incorporating a DNA Fragment Containing a Gene Coding for an Insecticidal Protein, Plasmids, Transformed Cyanobacteria Expressing Such Protein and Method for Use as a Biocontrol Agent" as unpatentable under 35 U.S.C. § 103, as well as the rejection of claims 1–48 and 50–51 under 35 U.S.C. § 112, first paragraph, for lack of enablement. We reverse the § 103 rejection. The § 112 rejection is affirmed in part and reversed in part.

BACKGROUND

A. The Invention

The claimed invention is directed to the use of genetic engineering techniques [1] for production of proteins that are toxic to insects such as larvae of mosquitos and black flies. These swamp-dwelling pests are the source of numerous human health problems, including malaria. It is known that certain species of the naturally-occurring *Bacillus* genus of bacteria produce proteins ("endotoxins") that are toxic to these insects. Prior art methods of combatting the insects involved spreading or spraying crystalline spores of the insecticidal *Bacillus* proteins over swamps. The spores were environmentally unstable, however, and would often sink to the bottom of a swamp before being consumed, thus rendering this method prohibitively expensive. Hence the need for a lower-cost method of producing the insecticidal *Bacillus* proteins in high volume, with application in a more stable vehicle.

As described by appellants, the claimed subject matter meets this need by providing for the production of the insecticidal *Bacillus* proteins within host cyanobacteria. Although both cyanobacteria and bacteria are members of the procaryote [2] kingdom, the cyanobacteria (which in the past have been referred to as "blue-green algae") are unique among procaryotes in that the cyanobacteria are capable of oxygenic photosynthesis. The cyanobacteria grow on top of swamps where they are consumed by mosquitos and black flies. Thus, when *Bacillus* proteins are produced with-

1. Basic vocabulary and techniques for gene cloning and expression have been described in *In re O'Farrell*, 853 F.2d 894, 895–99, 7 U.S.P.Q.2d 1673, 1674–77 (Fed.Cir.1988), and are not repeated here.

2. All living cells can be classified into one of two broad groups, procaryotes and eucaryotes. The procaryotes comprise organisms formed of cells that do not have a distinct nucleus; their DNA floats throughout the cellular cytoplasm. In contrast, the cells of eucaryotic organisms such as man, other animals, plants, protozoa, algae and yeast have a distinct nucleus wherein their DNA resides.

in transformed[3] cyanobacterial hosts according to the claimed invention, the presence of the insecticide in the food of the targeted insects advantageously guarantees direct uptake by the insects.

More particularly, the subject matter of the application on appeal includes a chimeric (i.e., hybrid) gene comprising (1) a gene derived from a bacterium of the *Bacillus* genus whose product is an insecticidal protein, united with (2) a DNA promoter effective for expressing[4] the *Bacillus* gene in a host cyanobacterium, so as to produce the desired insecticidal protein.

The claims on appeal are 1–48 and 50–52, all claims remaining in the application. Claim 1 reads:

> 1. A chimeric gene capable of being expressed in Cyanobacteria cells comprising:
>
> (a) a DNA fragment comprising a promoter region which is effective for expression of a DNA fragment in a Cyanobacterium; and
>
> (b) at least one DNA fragment coding for an insecticidally active protein produced by a Bacillus strain, or coding for an insecticidally active truncated form of the above protein or coding for a protein having substantial sequence homology to the active protein,
>
> the DNA fragments being linked so that the gene is expressed.

Claims 2–15, which depend from claim 1, recite preferred *Bacillus* species, promoters, and selectable markers.[5] Independent claim 16 and claims 17–31 which depend therefrom are directed to a hybrid plasmid vector which includes the chimeric gene of claim 1. Claim 32 recites a bacterial strain. Independent claim 33 and claims 34–48 which depend therefrom recite a cyanobacterium which expresses the chimeric gene of claim 1. Claims 50–51 recite an insecticidal composition. Claim 52 recites a particular plasmid that appellants have deposited.

B. Appellants' Disclosure

In addition to describing the claimed invention in generic terms, appellants' specification discloses two particular species of *Bacillus* (*B. thuringiensis, B. sphaericus*) as sources of insecticidal protein; and nine genera of cyanobacteria (*Synechocystis, Anacystis, Synechococcus, Agmenellum, Aphanocapsa, Gloecapsa, Nostoc, Anabaena* and *Ffremyllia*) as useful hosts.

The working examples relevant to the claims on appeal detail the transformation of a single strain of cyanobacteria, i.e., *Synechocystis* 6803. In one example, *Synechocystis* 6803 cells are transformed with a plasmid comprising (1) a gene encoding a particular insecticidal protein ("B.t. 8") from *Bacillus thuringiensis* var. *israelensis*, linked to (2) a particular promoter, the P_L promoter from the bacteriophage Lambda (a virus of *E. coli*). In another example, a different promoter, i.e., the *Synechocystis* 6803 promoter for the rubisco operon, is utilized instead of the Lambda P_L promoter.

C. The Prior Art

A total of eleven prior art references were cited and applied, in various combinations, against the claims on appeal.

The focus of Dzelzkalns,[6] the primary reference cited against all of the rejected claims, is to determine whether chloroplast promoter sequences can function in cyanobacteria. To that end Dzelzkalns discloses the expression in cyanobacteria of a chimeric gene comprising a chloroplast promot-

3. "Transformed" cyanobacteria are those that have successfully taken up the foreign *Bacillus* DNA such that the DNA information has become a permanent part of the host cyanobacteria, to be replicated as new cyanobacteria are generated.

4. "Expression" of a gene refers to the production of the protein which the gene encodes; more specifically, it is the process of transferring information from a gene (which consists of DNA) via messenger RNA to ribosomes where a specific protein is made.

5. In the context of the claimed invention, "selectable markers" or "marker genes" refer to antibiotic-resistance conferring DNA fragments, attached to the gene being expressed, which facilitate the selection of successfully transformed cyanobacteria.

6. 12 *Nucleic Acids Res.* 8917 (1984).

er sequence fused to a gene encoding the enzyme chloramphenicol acetyl transferase (CAT).[7] Importantly, Dzelzkalns teaches the use of the CAT gene as a "marker" gene; this use of antibiotic resistance-conferring genes for selection purposes is a common technique in genetic engineering.

Sekar I,[8] Sekar II,[9] and Ganesan [10] collectively disclose expression of genes encoding certain *Bacillus* insecticidal proteins in the bacterial hosts *B. megaterium, B. subtilis* and *E. coli.*

Friedberg [11] discloses the transformation of the cyanobacterium *Anacystis nidulans* R2 by a plasmid vector comprising the O_LP_L operator-promoter region and a temperature-sensitive repressor gene of the bacteriophage Lambda. While the cyanobacteria are attractive organisms for the cloning of genes involved in photosynthesis, Friedberg states, problems may still be encountered such as suboptimal expression of the cloned gene, detrimental effects on cell growth of overexpressed, highly hydrophobic proteins, and rapid turnover of some gene products. To address these problems, Friedberg teaches the use of the disclosed Lambda regulatory signals in plasmid vehicles which, it states, have "considerable potential for use as vectors the expression of which can be controlled in *Anacystis*...."

Miller [12] compares the initiation specificities *in vitro* of DNA-dependent RNA polymerases [13] purified from two different species of cyanobacteria (*Fremyella diplosiphon* and *Anacystis nidulans*), as well as from *E. coli.*

Nierzwicki-Bauer [14] identifies in the cyanobacterium *Anabaena* 7120 the start site for transcription of the gene encoding *rbcL,* the large subunit of the enzyme ribulose–1,5–bisphosphate carboxylase. It reports that the nucleotide sequence 14–8 base pairs preceding the transcription start site "resembles a good *Escherichia coli* promoter," but that the sequence 35 base pairs before the start site does not.

Chauvat [15] discloses host-vector systems for gene cloning in the cyanobacterium *Synechocystis* 6803, in which the antibiotic resistance-conferring *neo* gene is utilized as a selectable marker.

Reiss [16] studies expression in *E. coli* of various proteins formed by fusion of certain foreign DNA sequences with the *neo* gene.

Kolowsky [17] discloses chimeric plasmids designed for transformation of the cyanobacterium *Synechococcus* R2, comprising an antibiotic-resistant gene linked to chromosomal DNA from the *Synechococcus* cyanobacterium.

Barnes, United States Patent No. 4,695,-455, is directed to the treatment with stabilizing chemical reagents of pesticides produced by expression of heterologous genes (such as those encoding *Bacillus* proteins) in host microbial cells such as *Pseudomonas* bacteria. The host cells are killed by this treatment, but the resulting pesticidal compositions exhibit prolonged toxic activity when exposed to the environment of target pests.

7. Chloramphenicol is an antibiotic; CAT is an enzyme which destroys chloramphenicol and thus imparts resistance thereto.

8. 137 *Biochem. and Biophys. Res. Comm.* 748 (1986).

9. 33 *Gene* 151 (1985).

10. 189 *Mol. Gen. Genet.* 181 (1983).

11. 203 *Mol. Gen. Genet.* 505 (1986).

12. 140 *J. Bacteriology* 246 (1979).

13. RNA polymerase, the enzyme responsible for making RNA from DNA, binds at specific nucleotide sequences (promoters) in front of genes in DNA, and then moves through the gene making an RNA molecule that includes the information contained in the gene. Initiation specificity is the ability of the RNA polymerase to initiate this process specifically at a site(s) on the DNA template.

14. 81 *Proc. Natl. Acad. Sci. USA* 5961 (1984).

15. 204 *Mol. Gen. Genet.* 185 (1986).

16. 30 *Gene* 211 (1984).

17. 27 *Gene* 289 (1984).

D. The Grounds of Rejection

1. The § 103 Rejections

Claims 1–6, 16–21, 33–38, 47–48 and 52 (which include all independent claims in the application) were rejected as unpatentable under 35 U.S.C. § 103 based upon Dzelzkalns in view of Sekar I or Sekar II and Ganesan. The examiner stated that Dzelzkalns discloses a chimeric gene capable of being highly expressed in a cyanobacterium, said gene comprising a promoter region effective for expression in a cyanobacterium operably linked to a structural gene encoding CAT. The examiner acknowledged that the chimeric gene and transformed host of Dzelzkalns differ from the claimed invention in that the former's structural gene encodes CAT rather than insecticidally active protein. However, the examiner pointed out, Sekar I, Sekar II, and Ganesan teach genes encoding insecticidally active proteins produced by *Bacillus*, and the advantages of expressing such genes in heterologous [18] hosts to obtain larger quantities of the protein. The examiner contended that it would have been obvious to one of ordinary skill in the art to substitute the *Bacillus* genes taught by Sekar I, Sekar II, and Ganesan for the CAT gene in the vectors of Dzelzkalns in order to obtain high level expression of the *Bacillus* genes in the transformed cyanobacteria. The examiner further contended that it would have been obvious to use cyanobacteria as heterologous hosts for expression of the claimed genes due to the ability of cyanobacteria to serve as transformed hosts for the expression of heterologous genes. In the absence of evidence to the contrary, the examiner contended, the invention as a whole was prima facie obvious.

Additional rejections were entered against various groups of dependent claims which we need not address here. All additional rejections were made in view of Dzelzkalns in combination with Sekar I, Sekar II, and Ganesan, and further in view of other references discussed in Part C above.

The Board affirmed the § 103 rejections, basically adopting the examiner's Answer as its opinion while adding a few comments. The legal conclusion of obviousness does not require absolute certainty, the Board added, but only a reasonable expectation of success, citing *In re O'Farrell*, 853 F.2d 894, 7 U.S.P.Q.2d 1673 (Fed. Cir.1988). In view of the disclosures of the prior art, the Board concluded, one of ordinary skill in the art would have been motivated by a reasonable expectation of success to make the substitution suggested by the examiner.

2. The § 112 Rejection

The examiner also rejected claims 1–48 and 50–51 under 35 U.S.C. § 112, first paragraph, on the ground that the disclosure was enabling only for claims limited in accordance with the specification as filed. Citing *Manual of Patent Examining Procedure* (MPEP) provisions 706.03(n) [19] and (z) [20] as support, the examiner took the position that undue experimentation would be required of the art worker to practice the claimed invention, in view of the unpredictability in the art, the breadth of the claims, the limited number of working examples and the limited guidance provided

18. Denotes different species or organism.

19. MPEP 706.03(n), "Correspondence of Claim and Disclosure," provides in part:

> In chemical cases, a claim may be so broad as to not be supported by [the] disclosure, in which case it is rejected as unwarranted by the disclosure....

20. MPEP 706.03(z), "Undue Breadth," provides in part:

> [I]n applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. *In re Sol*, 1938 C.D. 723; 497 O.G. 546. This is because in arts such as chemistry it is not obvious from the disclosure of one species, what other species will work. *In re Dreshfield*, 1940 C.D. 351; 518 O.G. 255 gives this general rule: "It is well settled that in cases involving chemicals and chemical compounds, which differ radically in their properties it must appear in an applicant's specification either by the enumeration of a sufficient number of the members of a group or by other appropriate language, that the chemicals or chemical combinations included in the claims are capable of accomplishing the desired result." ...

in the specification. With respect to unpredictability, the examiner stated that

> [t]he cyanobacteria comprise a large and diverse group of photosynthetic bacteria including large numbers of species in some 150 different genera including *Synechocystis, Anacystis, Synechococcus, Agmenellum, Nostoc, Anabaena,* etc. The molecular biology of these organisms has only recently become the subject of intensive investigation and this work is limited to a few genera. Therefore the level of unpredictability regarding heterologous gene expression in this large, diverse and relatively poorly studied group of procaryotes is high....

The Board affirmed, noting that "the limited guidance in the specification, considered in light of the relatively high degree of unpredictability in this particular art, would not have enabled one having ordinary skill in the art to practice the broad scope of the claimed invention without undue experimentation. *In re Fisher,* 427 F.2d 833, 166 U.S.P.Q. 18 (CCPA 1970)."

OPINION

A. *Obviousness*

[1] We first address whether the PTO erred in rejecting the claims on appeal as prima facie obvious within the meaning of 35 U.S.C. § 103. Obviousness is a legal question which this court independently reviews, though based upon underlying factual findings which we review under the clearly erroneous standard. *In re Woodruff,* 919 F.2d 1575, 1577, 16 U.S.P.Q.2d 1934, 1935 (Fed.Cir.1990).

[2] Where claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, *inter alia,* consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *See In re Dow Chemical Co.,* 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531 (Fed.Cir.1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure. *Id.*

[3] We agree with appellants that the PTO has not established the prima facie obviousness of the claimed subject matter. The prior art simply does not disclose or suggest the expression in cyanobacteria of a chimeric gene encoding an insecticidally active protein, or convey to those of ordinary skill a reasonable expectation of success in doing so. More particularly, there is no suggestion in Dzelzkalns, the primary reference cited against all claims, of substituting in the disclosed plasmid a structural gene encoding *Bacillus* insecticidal proteins for the CAT gene utilized for selection purposes. The expression of antibiotic resistance-conferring genes in cyanobacteria, without more, does not render obvious the expression of unrelated genes in cyanobacteria for unrelated purposes.

The PTO argues that the substitution of insecticidal *Bacillus* genes for CAT marker genes in cyanobacteria is suggested by the secondary references Sekar I, Sekar II, and Ganesan, which collectively disclose expression of genes encoding *Bacillus* insecticidal proteins in two species of host *Bacillus* bacteria (*B. megaterium* and *B. subtilis*) as well as in the bacterium *E. coli.* While these references disclose expression of *Bacillus* genes encoding insecticidal proteins in certain transformed *bacterial* hosts, nowhere do these references disclose or suggest expression of such genes in transformed *cyanobacterial* hosts.

To remedy this deficiency, the PTO emphasizes similarity between bacteria and cyanobacteria, namely, that these are both procaryotic organisms, and argues that this fact would suggest to those of ordinary skill the use of cyanobacteria as hosts for expression of the claimed chimeric genes. While it is true that bacteria and cyanobacteria are now both classified as procaryotes, that fact alone is not sufficient to motivate the art worker as the PTO con-

tends. As the PTO concedes, cyanobacteria and bacteria are not identical; they are classified as two separate divisions of the kingdom Procaryotae.[21] Moreover, it is only in recent years that the biology of cyanobacteria has been clarified, as evidenced by references in the prior art to "blue-green algae." Such evidence of recent uncertainty regarding the biology of cyanobacteria tends to rebut, rather than support, the PTO's position that one would consider the cyanobacteria effectively interchangeable with bacteria as hosts for expression of the claimed gene.

At oral argument the PTO referred to additional secondary references, not cited against any independent claim (i.e., Friedberg, Miller, and Nierzwicki-Bauer), which it contended disclose certain amino acid sequence homology between bacteria and cyanobacteria. The PTO argued that such homology is a further suggestion to one of ordinary skill to attempt the claimed invention. We disagree. As with the Dzelzkalns, Sekar I, Sekar II, and Ganesan references discussed above, none of these additional references disclose or suggest that cyanobacteria could serve as hosts for expression of genes encoding *Bacillus* insecticidal proteins. In fact, these additional references suggest as much about *differences* between cyanobacteria and bacteria as they do about similarities. For example, Nierzwicki-Bauer reports that a certain nucleotide sequence (i.e., the -10 consensus sequence) in a particular cyanobacterium resembles an *E. coli* promoter, but that another nearby nucleotide sequence (the -35 region) does not. While Miller speaks of certain promoters of the bacteriophage Lambda that are recognized by both cyanobacterial and *E. coli* RNA polymerases, it also discloses that these promoters exhibited differing strengths when exposed to the different polymerases. Differing sensitivities of the respective polymerases to an inhibitor are also disclosed, suggesting differences in the structures of the initiation complexes.

The PTO asks us to agree that the prior art would lead those of ordinary skill to conclude that cyanobacteria are attractive hosts for expression of any and all heterologous genes. Again, we can not. The relevant prior art does indicate that cyanobacteria are attractive hosts for expression of both native and heterologous *genes involved in photosynthesis* (not surprisingly, for the capability of undergoing oxygenic photosynthesis is what makes the cyanobacteria unique among procaryotes). However, these references do not suggest that cyanobacteria would be equally attractive hosts for expression of *unrelated* heterologous genes, such as the claimed genes encoding *Bacillus* insecticidal proteins.

In *O'Farrell*, this court affirmed an obviousness rejection of a claim to a method for producing a "predetermined protein in a stable form" in a transformed bacterial host. 853 F.2d at 895, 7 U.S.P.Q.2d at 1674. The cited references included a prior art publication (the Polisky reference) whose three authors included two of the three coinventor-appellants. The main difference between the prior art and the claim at issue was that in Polisky, the heterologous gene was a gene for ribosomal RNA, while the claimed invention substituted a gene coding for a predetermined protein. *Id.* at 901, 7 U.S.P.Q.2d at 1679. Although, as the appellants therein pointed out, the ribosomal RNA gene is not normally translated into protein, Polisky mentioned preliminary evidence that the transcript of the ribosomal RNA gene was translated into protein, and further predicted that if a gene coding for a protein were to be substituted, extensive translation might result. *Id.* We thus affirmed, explaining that

> the prior art explicitly suggested the substitution that is the difference between the claimed invention and the prior art, and presented preliminary evidence suggesting that the [claimed] method could be used to make proteins.
>
> ....

21. *Stedman's Medical Dictionary* 1139 (24th ed. 1982) (definition of "Procaryotae"). Procaryotic organisms are commonly classified according to the following taxonomic hierarchy: Kingdom; Division; Class; Order; Family; Genus; Species. 3 *Bergey's Manual of Systematic Bacteriology* 1601 (1989).

> . . . Polisky contained detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting that it would be successful.

Id. at 901–02, 7 U.S.P.Q.2d at 1679–80.

In contrast with the situation in *O'Farrell*, the prior art in this case offers no suggestion, explicit or implicit, of the substitution that is the difference between the claimed invention and the prior art. Moreover, the "reasonable expectation of success" that was present in *O'Farrell* is not present here. Accordingly, we reverse the § 103 rejections.

B. Enablement

[4] The first paragraph of 35 U.S.C. § 112 requires, *inter alia*, that the specification of a patent enable any person skilled in the art to which it pertains to make and use the claimed invention. Although the statute does not say so, enablement requires that the specification teach those in the art to make and use the invention without "undue experimentation." *In re Wands*, 858 F.2d 731, 737, 8 U.S.P.Q.2d 1400, 1404 (Fed.Cir.1988). That *some* experimentation may be required is not fatal; the issue is whether the amount of experimentation required is "undue." *Id.* at 736–37, 8 U.S.P.Q.2d at 1404. Enablement, like obviousness, is a question of law which we independently review, although based upon underlying factual findings which we review for clear error. *See id.* at 735, 8 U.S.P.Q.2d at 1402.

[5] In response to the § 112 rejection, appellants assert that their invention is "pioneering," and that this should entitle them to claims of broad scope. Narrower claims would provide no real protection, appellants argue, because the level of skill in this art is so high, art workers could easily avoid the claims. Given the disclosure in their specification, appellants contend that any skilled microbiologist could construct vectors and transform many different cyanobacteria, using a variety of promoters and *Bacillus* DNA, and could easily determine whether or not the active *Bacillus* protein was successfully expressed by the cyanobacteria.

The PTO made no finding on whether the claimed invention is indeed "pioneering," and we need not address the issue here. With the exception of claims 47 and 48, the claims rejected under § 112 are not limited to any particular genus or species of cyanobacteria. The PTO's position is that the cyanobacteria are a diverse and relatively poorly studied group of organisms, comprising some 150 different genera, and that heterologous gene expression in cyanobacteria is "unpredictable." Appellants have not effectively disputed these assertions. Moreover, we note that only one particular species of cyanobacteria is employed in the working examples of appellants' specification, and only nine genera of cyanobacteria are mentioned in the entire document.

Taking into account the relatively incomplete understanding of the biology of cyanobacteria as of appellants' filing date, as well as the limited disclosure by appellants of particular cyanobacterial genera operative in the claimed invention, we are not persuaded that the PTO erred in rejecting claims 1–46 and 50–51 under § 112, first paragraph. There is no reasonable correlation between the narrow disclosure in appellants' specification and the broad scope of protection sought in the claims encompassing gene expression in any and all cyanobacteria. *See In re Fisher*, 427 F.2d 833, 839, 166 U.S.P.Q. 18, 24 (CCPA 1970) (the first paragraph of § 112 requires that the scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification).[22] Accordingly,

22. The enablement rejection in this case was not based upon a post-filing date state of the art, as in *In re Hogan*, 559 F.2d 595, 605–07, 194 U.S.P.Q. 527, 536–38 (CCPA 1977). *See also United States Steel Corp. v. Phillips Petroleum Co.*, 865 F.2d 1247, 1251, 9 U.S.P.Q.2d 1461, 1464 (Fed.Cir.1989) (citing *Hogan*); *Hormone Research Found., Inc. v. Genentech, Inc.*, 904 F.2d 1558, 1568–69, 15 U.S.P.Q.2d 1039, 1047–48 (Fed.Cir.1990) (directing district court, on remand, to consider effect of *Hogan* and *United States Steel* on the enablement analysis of *Fisher*), *cert. dismissed*, — U.S. —, 111 S.Ct. 1434, 113 L.Ed.2d 485 (1991). We therefore do not

we affirm the § 112 rejection as to those claims.

[6] In so doing we do *not* imply that patent applicants in art areas currently denominated as "unpredictable" must never be allowed generic claims encompassing more than the particular species disclosed in their specification. It is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. *In re Angstadt*, 537 F.2d 498, 502–03, 190 U.S.P.Q. 214, 218 (CCPA 1976). However, there must be sufficient disclosure, either through illustrative examples or terminology,[23] to teach those of ordinary skill how to make and how to use the invention as broadly as it is claimed. This means that the disclosure must adequately guide the art worker to determine, without undue experimentation, which species among all those encompassed by the claimed genus possess the disclosed utility. Where, as here, a claimed genus represents a diverse and relatively poorly understood group of microorganisms, the required level of disclosure will be greater than, for example, the disclosure of an invention involving a "predictable" factor such as a mechanical or electrical element. *See Fisher*, 427 F.2d at 839, 166 U.S.P.Q. at 24. In this case, we agree with the PTO that appellants' limited disclosure does not enable one of ordinary skill to make and use the invention as now recited in claims 1–46 and 50–51 without undue experimentation.

Remaining dependent claim 47 recites a cyanobacterium which expresses the chimeric gene of claim 1, wherein the cyanobacterium is selected from among the genera *Anacystis* and *Synechocystis*. Claim 48, which depends from claim 47, is limited to the cyanobacterium *Synechocystis* 6803. The PTO did not separately address these claims, nor indicate why they should be treated in the same manner as the claims encompassing all types of cyanobacteria. Although these claims are not limited to expression of genes encoding particular *Bacillus* proteins, we note what appears to be an extensive understanding in the prior art of the numerous *Bacillus* proteins having toxicity to various insects. The rejection of claims 47–48 under § 112 will not be sustained.

consider the effect of *Hogan* and its progeny on *Fisher's* analysis of when an inventor should be allowed to "dominate the future patentable inventions of others." *Fisher*, 427 F.2d at 839, 166 U.S.P.Q. at 24.

23. The first paragraph of § 112 requires nothing more than *objective* enablement. *In re Marzocchi*, 439 F.2d 220, 223, 169 U.S.P.Q. 367, 369 (CCPA 1971). How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is irrelevant. *Id.*

CONCLUSION

The rejection of claims 1–48 and 50–52 under 35 U.S.C. § 103 is *reversed*. The rejection of claims 1–46 and 50–51 under 35 U.S.C. § 112, first paragraph, is *affirmed* and the rejection of claims 47 and 48 thereunder is *reversed*.

AFFIRMED-IN-PART, REVERSED-IN-PART.

MAYER, Circuit Judge, dissenting.

An appeal is not a second opportunity to try a case or prosecute a patent application, and we should not allow parties to "undertake to retry the entire case on appeal." *Perini America, Inc. v. Paper Converting Machine Co.*, 832 F.2d 581, 584, 4 U.S.P.Q.2d 1621, 1624 (Fed.Cir.1987); *Eaton Corp. v. Appliance Valves Corp.*, 790 F.2d 874, 877, 229 U.S.P.Q. 668, 671 (Fed. Cir.1986). But that is precisely what the court has permitted here. The PTO conducted a thorough examination of the prior art surrounding this patent application and concluded the claims would have been obvious. The board's decision based on the examiner's answer which comprehensively explains the rejection is persuasive and shows how the evidence supports the legal conclusion that the claims would have been obvious. Yet, the court ignores all this and conducts its own examination, if you will, as though the examiner and board did not exist. Even if I thought this opinion were more persuasive than the board's, I could

not join it because it misperceives the role of the court.

The scope and content of the prior art, the similarity between the prior art and the claims, the level of ordinary skill in the art, and what the prior art teaches are all questions of fact. *Graham v. John Deere Co.*, 383 U.S. 1, 17, 86 S.Ct. 684, 693–94, 15 L.Ed.2d 545, 148 U.S.P.Q. 459, 467 (1966); *Jurgens v. McKasy*, 927 F.2d 1552, 1560, 18 U.S.P.Q.2d 1031, 1037 (Fed.Cir.1991). And "[w]here there are two permissible views of the evidence, the factfinder's choice between them cannot be clearly erroneous." *Anderson v. City of Bessemer City*, 470 U.S. 564, 574, 105 S.Ct. 1504, 1511–12, 84 L.Ed.2d 518 (1985). The mere denomination of obviousness as a question of law does not give the court license to decide the factual matters afresh and ignore the requirement that they be respected unless clearly erroneous. *In re Woodruff*, 919 F.2d 1575, 1577, 16 U.S.P.Q.2d 1934, 1935 (Fed.Cir.1990); *In re Kulling*, 897 F.2d 1147, 1149, 14 U.S.P.Q.2d 1056, 1057 (Fed. Cir.1990). There may be more than one way to look at the prior art, but on this record we are bound by the PTO's interpretation of the evidence because it is not clearly erroneous and its conclusion is unassailable. I would affirm on that basis.

LEVERNIER CONSTRUCTION, INC., Plaintiff-Appellee,

v.

The UNITED STATES, Defendant-Appellant.

No. 91-5058.

United States Court of Appeals, Federal Circuit.

Oct. 22, 1991.

Construction contractor sought attorney fees and expenses under the Equal Access to Justice Act (EAJA) after settlement of equitable adjustment claim. On original hearing, the Claims Court, Reginald W. Gibson, J., 21 Cl.Ct. 683, granted application in part and denied it in part. Contractor sought reconsideration. The Claims Court, 22 Cl.Ct. 247, granted the motion, and held that contractor was entitled to recover additional amount representing consultant fees and expenses. Government appealed. The Court of Appeals, Bennett, Senior Circuit Judge, held that: (1) prosecution of equitable adjustment claim before contracting officer was not a "civil action" within meaning of the EAJA, and thus contractor was not entitled to recover consultant fees incurred in preparation of equitable adjustment claim; (2) Claims Court erred in applying 18% cost of living adjustment (COLA) to paralegal fees awarded under the EAJA; and (3) it was error to apply 18% (COLA) to hourly rates of attorneys whose time was claimed at $75 an hour.

Reversed.

1. United States ⇐147(12)

Prosecution of equitable adjustment claim before contracting officer was not "civil action" within meaning of the Equal Access to Justice Act (EAJA), and thus contractor was not entitled to recover fees incurred by contract claim consultant for preparation of equitable adjustment claim. 28 U.S.C.A. § 2412.

See publication Words and Phrases for other judicial constructions and definitions.

2. United States ⇐147(5)

Equal Access to Justice Act (EAJA) is a waiver of sovereign immunity which must be strictly construed. 28 U.S.C.A. § 2412.

3. United States ⇐147(4)

In formulating an award of attorney fees under the Equal Access to Justice Act (EAJA), court may adjust statutory cap governing rate of attorney fees upward to account for an increase in cost of living. 28 U.S.C.A. § 2412(d)(2)(A)(ii).